লাল-সবুজে দাগানো

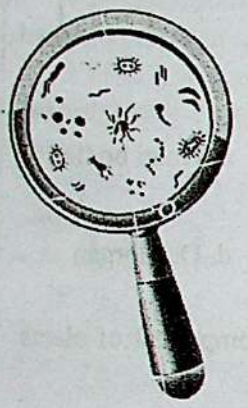
TEXT BOOK

Botany

UNMESH

Medical & Dental Admission Care

4 Chapter Four
Microbes



Microbes are everywhere, but we cannot see them. They make us sick, but we can't live without them. Microbes are important part in our earth's natural environment, but we understand so little about them. A microbe is any living thing that spends its life at a size visible sometimes only with a microscope. The viruses, bacteria and protozoans are considered as microbes. Microbiology is the science deals with microbes. Antony van Leeuwenhoek regarded as the father of microbiology. In this chapter a discussion has been made about the structure, life cycle and disease relating activities of microbes like virus, bacteria and malaria parasite.

Key words: Virus, hepatitis, ring spot of papaya, bacteriophage, dengue, cholera, blight diseases, malaria.

Period 15: After reading this chapter students should be able to (Learning output) -
❒ Describe the characteristics, structure and importance of virus.
❒ Describe the diagrammatic life cycle of bacteriophage.
❒ Analyze the symptoms, remedy and prevention of viral diseases.
❒ Classify bacteria on the basis of their shape.
❒ Describe the structure and reproduction of bacteria.
❒ Explain the importance of bacteria.
❒ Find the symptoms and ways of prevention of bacterial diseases.
❒ **Practical:** Identify and draw the bacteria.
❒ Describe the life cycle of *Plasmodium vivax* (malaria parasite).
❒ Explain the infection methods and prevention ways of malaria parasite.

4.1 Viruses

The word virus has come from the Latin *virus* referring to poison and other noxious substances, first used in English in 1392. In ancient period any pathogenic poisonous substances are called as virus.

Definition

1. A virus is a small infectious agent that replicates only inside the living cells of other organisms and can infect all types of life forms, from animals and plants to microorganisms, including bacteria and archaea.

2. According to **Merriam-Webster Dictionary,** *virus is an extremely small living thing that causes a disease and that spreads from one person or animal to another.*

3. Viruses are very small, submicroscopic, infectious particles of protein and nucleic acid that produced significant and infectious diseases in plants and animals and can multiply within specific living hosts and exist as non-living substances outside of any biological entity.

Brief history

Viruses were not known to biologists for a long time due to their ultramicroscopic structure although their presence was apparent by infectious diseases which were not due to bacteria. They attracted the attention of investigators in the 19th century when mosaic disease caused severe damage to commercially important tobacco crop.

- Russian scientist **Dmitri Ivanovsky** in 1892 describing a non-bacterial pathogen infecting tobacco plants.
- In 1898, the Dutch microbiologist **Martinus Beijerinck** discovered the **tobacco mosaic virus** (TMV) from the tobacco plants.
- In 1901 **Walter Reed** first discovered the virus of yellow fever disease in human.
- The first images of viruses were obtained upon the invention of electron microscopy in 1931 by the German engineers **Ernst Ruska** and **Max Knoll.**
- American virologist, **Wendell Stanley** in 1935 isolated and crystallized TMV from the infested leaves of tobacco. For this contribution he was awarded the Nobel Prize in Chemistry in 1946.
- English scientists **F. C. Bawden** and **N. W. Pirie** in 1937 have proved that viruses are chemically nucleic acid and protein components.
- The first X-ray diffraction pictures of the crystallized virus were obtained by **Bernal** and **Fankuchen** in 1941. On the basis of her pictures, **Rosalind Franklin** discovered the full structure of the virus in 1955.
- In 1963, the hepatitis B virus was discovered by **Baruch Blumberg** and in 1965 **Howard Temin** described the first retrovirus.
- **Robert Gallo** and **Luc Montagnier** in 1984 independently isolated the retrovirus now called HIV that causes AIDS.
- In 1989 **Harvey J. Alter** discovered the hepatitis C virus.

From the beginning of **Ivanovsky's period** about 5,000 viruses have been described in detail, although there are millions of different types.

Virology: Virology is the science dealing with the study of viruses and the diseases caused by them. **W. M. Stanley** is known as the father of Virology.

General characteristics

A large number of viruses are now known. They exhibit diversity of form and infect a number of organisms. Despite diversity of form and structure, they show the following important characteristics common to all viruses:

1. They are ultramicroscopic entities.
2. They have no cellular organization and also no metabolic machinery of their own.
3. They are simple in structure, basically composed of nucleic acid wrapped up in a protein coat.
4. Nucleic acid is only of one type, either DNA or RNA, but never both.
5. They are obligatory intracellular parasites as they are completely inactive outside the host.
6. They multiply within the host by commandeering the metabolic machinery of the host cell.
7. They are specific in action, i.e. they always infect particular organ or organism.
8. They are incapable of growth and division.
9. They can be crystallized and even in crystalline form, they retain their infectivity.

10. They are unaffected by antimicrobial antibiotics.
11. They may undergo mutations.

Biological position of viruses

Viruses lack a cytoplasmic membrane and they do not have the basic component of a cell. They can only replicate inside the host cell. Outside the host cell, they are non-living. Thus, viruses show characters of both living and non-living.

Non-living characters of viruses: Following characters of viruses assign them as non-living:

1. They can be crystallized like an ordinary chemical and stored in a bottle or test tube indefinitely.
2. Outside the cell, they behave like inert chemicals.
3. They do not show growth, development, nutrition, reproduction, etc.
4. Sedimentation of viruses is according to their molecular weight like.
5. They do not have functional autonomy i.e. they are not capable of any function unless they obtain metabolic products from others.
6. Energy producing enzyme system is absent.

Living characters of viruses: Following characters of viruses assign them as living:

1. They multiply within host cells.
2. They possess genetic material, either DNA or RNA.
3. There have definite races or strains.
4. They exhibit mutations.
5. They bring about enzymatic changes *in vitro*.
6. They are able to infect and cause disease to living beings.
7. The DNA and proteins of viruses are similar in composition and structure to those of higher organisms.

Because of the above reasons, viruses form unique bridge between living and non-living things. Nobel laureate **A. Lwoff** (1953) identified virus as virus and defined them nor a living substance neither a non-living substance but in between them as individual existence. According to **Salle** (1974) the viruses are nothing but as chemicals in between the living cells.

Virus habitat

Viruses are found in almost every ecosystem on earth and are the most abundant type of biological entity. There are several viruses that have an animal or plant reserviour from where they affect humans. One milliliter of sea water contains 0.1 million virus.

Size and shape

Viruses are minute entities, even smaller than the smallest bacterium. They can be seen only under electron microscope as small particles called **virons**. Being minute, they are measured in millimicrons (1mm = 1/1000m). Generally, the plant viruses range in size from 17nm to 2000nm, while animal viruses range in size from 20- 350 nm.

Smallest Plant Virus: *Satellite Tobacco Necrosis virus*, 17 nm
Largest Plant Virus: *Citrus Triesteza virus*, 2000 × 12nm
Smallest Animal Virus: *Foot and mouth disease virus*, 20 nm

Largest Animal Virus: *Smallpoxvirus (Variola)*, 350 × 250 ×100 nm

Viruses have different shapes, as-

1. Rod-shaped virus: Tobacco mosaic virus (TMV), etc.

2. Spherical virus: HIV, Flavivirus.

3. Polygonal or cubical virus: Adeno virus, Vaccinia virus etc.

4. Tadpole shaped virus: Bacteriophage, T_2, T_4, T_6,

5. Cylindrical or thread shaped virus: Ebola virus.

6. Oval shaped virus: Influenza virus.

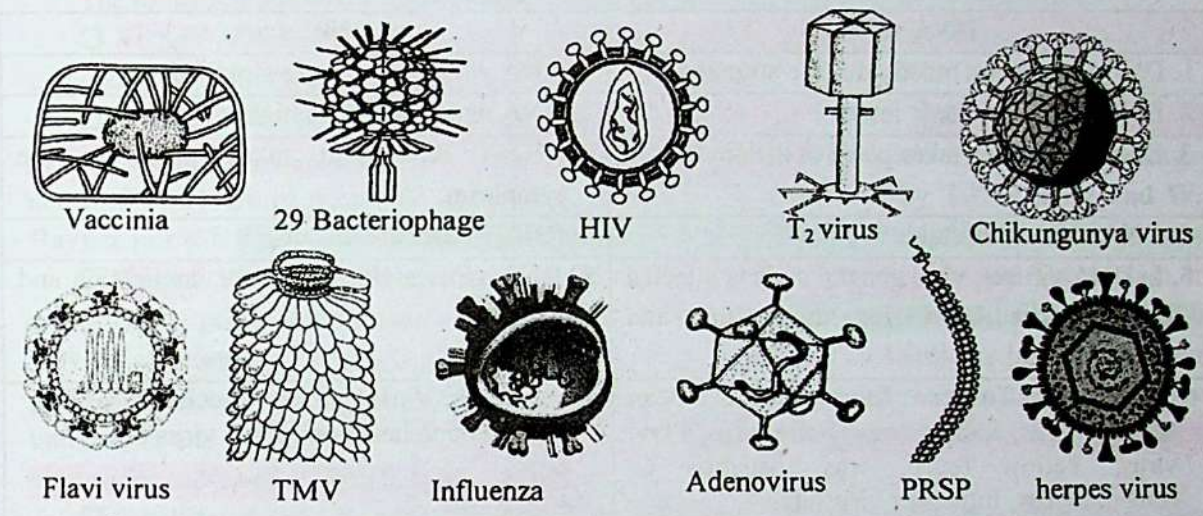


Fig 4.1 Different types of virus

Classification of viruses

A. According to the type of hosts they infect, viruses may be classified as one of the following three types:

1. Plant viruses: These are pathogenic viruses which infect plants. They are usually rod-shaped, containing nucleic acid in the form of RNA, e.g. tobacco mosaic virus (TMV), been yellow virus (BYV) etc.

2. Animal viruses: These are pathogenic viruses infecting animals. They are generally polyhedral or spherical in shape. The capsid in some is surrounded by envelope, and the nucleic acid is either DNA or RNA.

3. Bacteriophage: These are pathogenic viruses infecting bacteria and are called *bacteriophages* or simply *phages*. Their nucleic acid is DNA, e.g. T_2, T_4, T_6 bacteriophages. **Felix d'Herelle** in 1917 discovered bacteriophages.

B. Based on the genetic components viruses are of two types:

1. DNA virus: DNA virus is a virus that has DNA as its genetic material and replicates using a DNA-dependent *DNA polymerase*. The nucleic acid is usually double-stranded DNA (dsDNA) but may also be single-stranded DNA (ssDNA). These are the animal viruses and phages. Notable diseases like smallpox and chickenpox are caused by such DNA viruses.

2. RNA virus: RNA virus is a virus that has RNA (ribonucleic acid) as its genetic material. This nucleic acid is usually single-stranded RNA (ssRNA), but may be double-stranded RNA (dsRNA).

These are the plant viruses but notable human diseases caused by RNA viruses include SARS, influenza, hepatitis C, West Nile fever, polio and measles.

C. Other type

A virus that infects fungi is called **mycophage.** **Cyanophages** are viruses that infect cyanobacteria, also known as Cyanophyta or blue-green algae. **Holmes** (1948) designated virus as ***Phaginae*** that infects bacteria, as ***Phytophaginae*** that infects plant and as ***Zoophaginae*** that infects animals.

Differences between DNA virus and RNA virus

DNA virus	RNA virus
1. DNA viruses are mostly double-stranded	1. RNA viruses are single-stranded.
2. DNA mutation rate is lower	2. RNA mutation rate is higher
3. DNA replication takes place in the nucleus	3. RNA replication takes place in the cytoplasm.
4. DNA viruses are stable	4. RNA viruses are unstable.
5. In DNA viruses, viral genetic code is injected in the host DNA for duplication and decoding.	5. RNA viruses skip DNA for duplication and decoding.
6. Examples: Tobacco Mosaic Virus, Bean Mosaic Virus, Rabies virus, Polio virus, Flavi virus, Yellow fever virus, hepatitis C, Rubeola virus, Influenza virus etc.	6. Examples: Variola virus, Vaccinia virus, T_2 virus, Adeno herpes simplex virus etc.

Structure of Virus

The viral structure has three components:

1. Nucleic acid: The nucleic acid is in the central core. Unlike living organisms it contains a single molecule either of DNA or RNA, but never both. Nucleic acid is the only active part of a virus, hence viruses are sometimes called **wandering genes**. The infectivity of virus is due to nucleic acid while host specificity is determined by the protein coat.

2. Capsid: The capsid is the outer protective coat mostly made up of specific protein. It protects nucleic acid from inactivation by enzyme *nuclease* in the environment. It is often composed of many identical subunits called **capsomeres.** The shape and arrangement of capsomeres determine the shape of the virus. The capsid in close contact with nucleic acid, is known as **nucleocapsid.** Viruses may be enveloped or non-enveloped (naked).

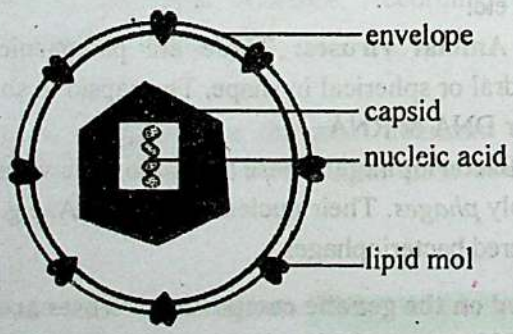


Figure 4.2 A generalized structure of virus

3. Envelope: Some viruses, for example influenza virus, mumps virus etc. acquire an outer lipoprotein coat by "budding" through the host cell membranes and are thus called **enveloped viruses.** The envelop contains host and viral proteins that are important for interaction with cellular components during the process of infection and replication. The host's defense mechanisms are directed against these viral antigenic epitopes.

Chemical composition of Virus

A virus is a nucleoprotein, i.e. mainly consisting of nucleic acid and proteins. Nucleic acid is either DNA or RNA, but never both. When only RNA is present, genetic information is solely carried by RNA, which is the unique property of virus. The envelope, if present, contains lipoproteins. The lipid is mostly derived from the host plasma membrane while the protein is virus coded. Viruses normally do not possess any biosynthetic enzymes.

Subviral agents

The following agents are smaller than viruses but have only some of their properties:

❒ **Virion:** A complete viral particle, consisting of RNA or DNA surrounded by a protein shell and constituting the infective form of a virus is called virion.

❒ **Viroid:** Viroids are the smallest infectious pathogens known, consisting solely of short strands of circular, single-stranded RNA without protein coats. They are mostly plant pathogens, some of which are of economic importance. Viroids were discovered by **T.D. Diener and W. S. Rayner** in 1967. The human pathogen hepatitis D virus is a defective RNA virus similar to viroids.

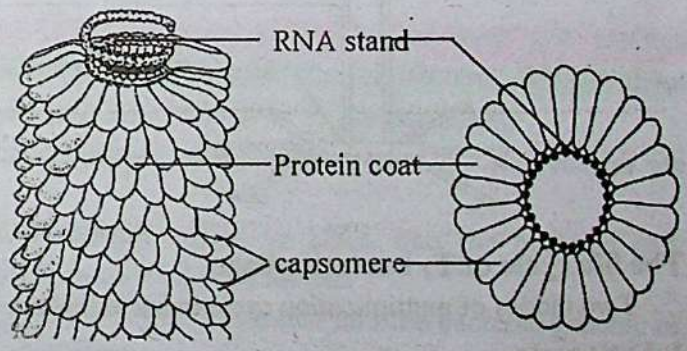
❒ **Prions:** A **prion** is an infectious agent, composed entirely of protein. The protein it is composed of prion protein called PrP, can fold in multiple. **Stanely B. Prusiner** discovered this very little microbe in 1982 and won the Nobel Prize in Physiology or Medicine in 1997. All known prion diseases in mammals affect the structure of the brain or other neural tissue and all are currently untreatable and universally fatal. This agent is responsible for ***Kuru*** disease of human, ***mad cow*** disease for cattle and ***scrapie*** disease for sheep.

❒ **Satellite:** A satellite is a subviral agent composed of **nucleic acid** that depends on the co-infection of a host cell with a **helper** or **master virus** for its replication. When a satellite subviral agent encodes the coat protein in which it is encapsulated, it is then called a **satellite virus.**

4.2 Tobacco Mosaic Virus (TMV)

Tobacco mosaic virus (TMV) is a virus that infects a wide range of plants, especially tobacco and other members of the family Solanaceae.

The infection causes characteristic patterns, such as "mosaic"-like mottling and discoloration on the leaves. TMV was the first virus ever to be discovered. In 1892, **Dmitri Ivanovsky** gave the first concrete evidence for the existence of a non-bacterial infectious agent for the tobacco mosaic disease. In 1898, Dutch microbiologist **Martinus Beijerinck** first discovered the TMV.



TS of TMV

Figure 4.3 TMV

Tobacco mosaic virus was the first virus to be crystallized. It was achieved by Wendell Meredith Stanley in 1935 and for this he was awarded the Nobel Prize in Chemistry in 1946. T appears like a hallow cylinder of 280-300 nanometer (nm) long and 15-18 nm thick. It c RNA core and protein coat. Proteins and RNA are interwined to form helical, grooved c

rods of uniform diameter with hollow channel of 20 Å wide. The protein coat of TMV is called **capsid** which built up of 2130 similar subunits strung around axial but helically oriented RNA from inside, in circular fashion. Each subunit of capsid is designated as **capsomere**. Each capsomere has a molecular weight of 17,400 and is formed by condensation of 158 aminoacids.

4.3 Bacteriophage or T_2 phage

T_2 virus is a more familiar virus. These are obligate intracellular parasites that multiply inside bacteria by making use of some or all of the host biosynthetic machinery and finally kill them, hence these are called as **bacteriophages.** T_2 only attacks susceptible bacterium known as *Escherichia coli* (*E. coli*). T_2 viruses were discovered by **Twort** (1915). **d. Heralle** (1917) named them as bacteriophage.

Structure: T_2 bacteriophage is a tadpole shaped virus with head and tail regions:

1. Head: The head is 93nm (nanometer) long and 65nm wide and has a hexagonal prism like appearance. The head contains a circular double stranded DNA covered by protein made bilayer capsid.

2. Tail: The tail is cylindrical and elongated structure behind the head. It is about 100 nm long with 25nm diameter. Tail is consists of a core tube which is surrounded by a contractile protein sheath. The sheath is connected to a thin disc **collar** at the upper end and a hexagonal **basal plate** at the lower end. From the each corner of the basal plate is given off a long, thin tail fiber and spike which help in attachment of the virus to its host cell.

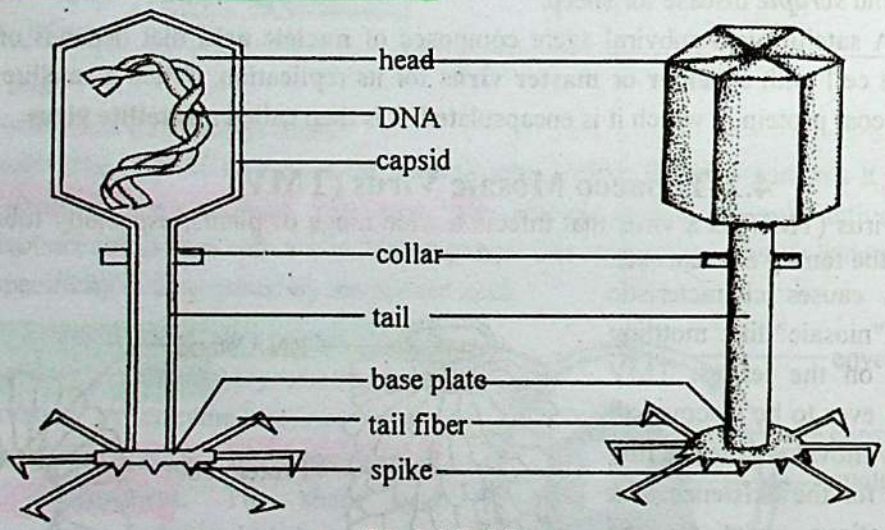


Fig 4.4 T_2 Virus

The life cycle of T_2 bacteriophage

Two modes of multiplication cycle in bacteriophage *viz.*, lytic cycle and lysogenic cycle.

1. Lytic cycle

Lytic cycle or lytic phages called as **virulent phages** multiplies inside the host bacterium and new viral particles comes out by lysing or by rupturing the host bacterial cell wall. e.g. T phages, T_2, T_4, T_6 etc. Lytic cycle completed by following stages:

(i) Adsorption: The phage virus attaches itself to the cell wall of the bacterium *E. coli* with the help of its tail fibers.

(ii) Penetration: Following adsorption, the phage injects its DNA into the bacterial cell. Injection of viral genome into the host through the hollow tubes of the tail.

(iii) Synthesis: Inside the host, the viral genome directs the synthesis of viral proteins using the machinery of the host. Viral genome generally encodes for some enzymes and coat proteins.

(iv) Multiplication: Viral genome replicates inside the host making several copies. Each viral genome gets packaged inside the protein coat. These intact mature infectious particles are called **virions.**

(v) Lysis: The crowding of virions inside the host ultimately causes cell lysis and l mature viral particles ~ about 200 mature phages/cells are liberated.

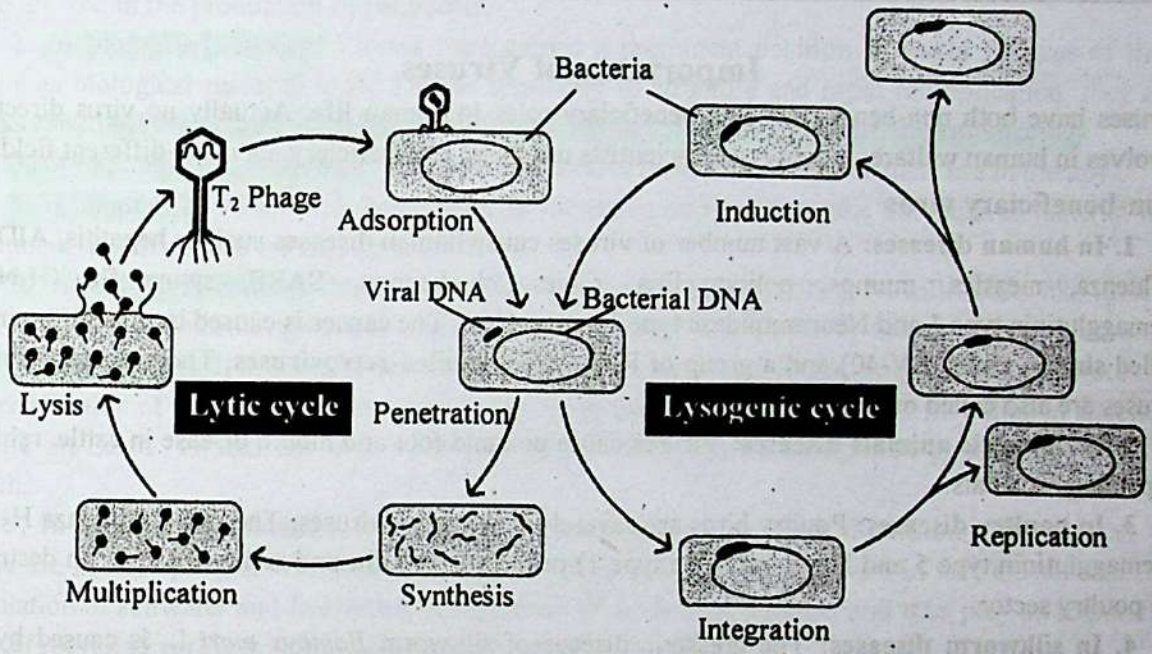


Fig 4.5 Life cycle of T_2 Phage

2. Lysogenic cycle

Lysogenic cycle or lysogenic phages called as **temperate phages** does not undergo multiplication or induce lysis, here the viral DNA gets integrated into the bacterial DNA without causing lysis. e.g. Lambda (λ) phages. Lysogenic cycle completed by following stages:

(i) Adsorption: Attachment of adsorption of tail fiber of the phage on to a specific receptor site on the bacterial cell wall.

(ii) Penetration: Following adsorption, the phage injects its DNA into the bacterial cell. Injection of viral genome into the host through the hollow tubes of the tail.

(iii) Integration: After the entry of viral genome, it gets integrated into the bacterial genome of the host. The viral genome integrated to the bacterial genome is termed **prophage.**

(iv) Replication: Viral genome replicates along with the bacterial genome replication and pass on to the daughter cells.

(v) Induction of lytic cycle: Occasionally, integrated viral genome detaches and ...ised into the bacterial cytoplasm. This dissociation is called induction and lytic cycle is followed releasing mature lysogenic phages.

Differences between Lytic and lysogenic cycle

Lytic cycle	Lysogenic cycle
1. Viral DNA destroys cell DNA, takes over cell functions and destroys the cell.	1. Viral DNA merges with cell DNA and does not destroy the cell.
2. The virus replicates and produces progeny phages.	2. The virus does not produce any progeny phages.
3. Prophage can not be seen.	3. Prophage' can only be seen.
4. The lytic cycle cannot be followed by the lysogenic cycle.	4. The lysogenic cycle is followed by the lytic cycle.
5. The lytic cycle can be seen in T phages virus types	5. The lysogenic cycle can be seen in Lambda (λ) phages virus types

Importance of Viruses

Viruses have both non-beneficiary and beneficiary roles in human life. Actually no virus directly involves in human welfare, in most cases scientists use them as beneficiary agents in different fields.

Non-beneficiary virus

1. In human diseases: A vast number of viruses cause human diseases such as hepatitis, AIDS, influenza, measles, mumps, poliomyelitis, rabies, chickenpox, SARS, swine flu (H_1N_1= Hemagglutinin type 1 and Neuraminidase type 1), cancer etc. The cancer is caused by the DNA virus called **simian virus** (SV-40) and a group of RNA viruses called **retroviruses**. The cancer causing-viruses are also called **oncogenic viruses.**

2. In domestic animals diseases: Viruses cause pox and foot and mouth disease in cattle, rabies in pet dogs and cats.

3. In poultry diseases: Poultry birds are severely infected by viruses. The avian influenza H_5N_1 (Hemagglutinin type 5 and Neuraminidase type 1) produces **bird flu** and **swine flu** and can destroy the poultry sector.

4. In silkworm diseases: The grasserie disease of silkworm *Bombyx mori* L. is caused by a nuclear polyhedrosis virus (NPV), sometime which is the only reason of declination of silk industry.

5. In plants diseases: A vast number of viruses cause plant diseases as rice tungro bacilliform virus (RTBV), tobacco mosaic virus (TMV), tomato/papaya ring spot virus, tomato leaf curl, potato leaf roll virus etc.

6. In reducing soil fertility: Apart from causing diseases in crops, bacteriophages attack the nitrogen fixing bacteria of the soil and are responsible for reducing fertility of the soil.

7. Epidemic and Pandemic virus: Some viruses are epidemic which spreads rapidly to many people and some viruses are pandemic which spread diseases worldwide.

- ***Human immune deficiency virus*** (HIV) is an example of one of the most destructive global pandemics in history that is responsible for ***acquired immune deficiency syndrome*** (AIDS) of human being.
- In 2003, the ***severe acute respiratory syndrome*** (SARS) epidemic took the lives of nearly 800 people worldwide.
- In 2014 ***Ebola virus disease*** (EVD) virus spread epidemically throughout the world.
- In 2016, **Zika disease** caused by *Zika virus* that is spread to people primarily through the bite of an infected *Aedes* species mosquito in Brazil.

■ In 2017, **Chikungunya fever** caused by *Chikungunya virus* (α- RNA type virus) that is spread to people primarily through the bite of infected *Aedes aegypti* and *Aedes albopictus* species mosquitoes in Bangladesh.

8. In weapons and biological warfare: Viruses may be tiny but have the capacity to cause death and devastation to large populations in epidemics and pandemics. This has led to the concern that viruses could be used for biological warfare.

Beneficiary viruses

1. In industry: Viruses are used in preparation of *sera* and *vaccines* to be used against diseases like rabies, polio, hepatitis B, papillomavirus etc. The multiplication of viruses in bacterial cell is also utilized in the production of antibodies.

2. In biological studies: Viruses have gained a prominent position in world because of their value as biological research tools. Due to simplicity of structure and rapid multiplication, they are widely used in research, in the fields of molecular biology, medicine and genetic engineering.

3. In bacteriophage therapy: Bacteriophages have been researched for their use in therapy.

4. In medicine: Viruses are being used as vectors or carriers that take the required material for treatment of a disease to various target cells.

5. In biological control: Viruses are used by humans in eradicating harmful pests like insects (by NPV) and in controlling the population of organisms such as rabbits by inducing *viral infection.*

8. In evolution: It is thought that viruses played a central role in the early evolution, before the diversification of bacteria, archaea and eukaryotes, at the time of the last universal common ancestor of life on Earth. Viruses are still one of the largest reservoirs of unexplored genetic diversity on Earth.

9. In aquatic ecosystems: A teaspoon of seawater contains about 1 million viruses. Most of these are bacteriophages, which are harmless to plants and animals, and are in fact essential to the regulation of saltwater and freshwater ecosystems. They destroy bacteria and thus play an important role of recycling carbon in the aquatic environment.

4.3 Viral diseases

Viral diseases in Animals

Name of viruses	Hosts	Name of diseases
Influenza virus	Human	Influenza
Herpes virus	Human	Herpes
Hepatites virus	Human	Jaundice
HIV	Human	AIDS
Variola virus	Human	Small pox
Rubeola virus	Human	Measles
Polio virus	Human	Polio
Rabies virus	Human	Rabies
Yellow fever virus	Human	Yellow fever
Flavi virus	Human	Dengue
Vaccinia virus	Cow	Cow-pox
Foot and mouth virus	Cow	Foot and mouth disease

Name of viruses	Hosts	Name of diseases
Viral diseases of Plants		
Papaya ringspot virus	Papaya	Papaya ring spot disease
Tobacco Mosaic Virus	Tobacco	Tobacco mosaic disease
Bean Mosaic Virus	Bean	Bean mosaic disease
Tungro Virus	Rice	Tungro disease of rice
Bushystant virus	Tomato	Bushystant disease of tomato
Banchy top virus	Banana	Banchy top disease banana

1. Ring spot disease of Papaya

Papaya ring spot is one of the most destructive diseases of papaya and occurs in nearly every region where papaya is grown except for Africa. It is particularly severe in areas of Thailand, Bangladesh, India, Taiwan, the Philippines, and the southern region of China. Additionally, the disease is widespread in the Caribbean islands and South America, and is found in the papaya growing areas of the U.S., including Florida, Hawaii, and Texas.

Causes of disease

This disease caused by *Papaya ringspot virus* (PRSV) in the genus *Potyvirus* and the virus family *Potyviridae*. The virus is a non-enveloped, flexous rod-shaped particle that is between 760–800 nm long and 12 nm in diameter. The virus (PRSV) causes ringspot disease of papaya and mosaic disease of several members of the melon family (Cucurbitaceae).

Infection

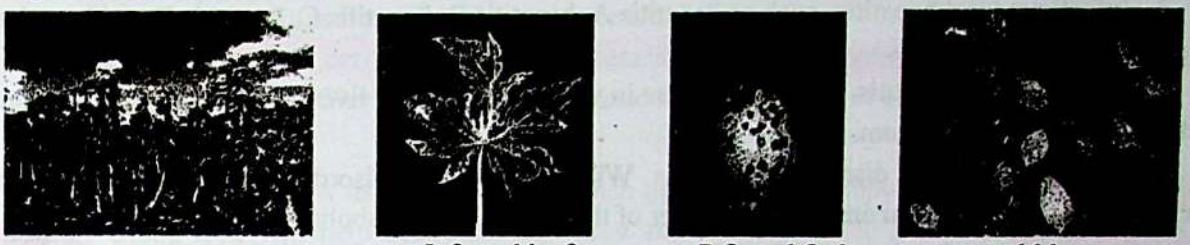
The virus, PRSV, is transmitted nonpersistently by peach aphid-*Myzus persicae* and melon aphid-*Aphis gossypii*. The disease cycle can start with aphids feeding on infected papaya for as little as 15 seconds and subsequently feeding on a healthy papaya. There is no incubation period. The virus does not persist in the vector so transmission to another plant has to occur rather rapidly. The amount of primary infection increases as the distance from infected papaya trees decrease. Secondary infection spreads rapidly and an orchard can become totally infected in three to four months. This situation occurs in young orchards located close to infected plants and during periods when populations of winged aphid flights are high.

Symptoms

Papaya ring spot virus infects papaya and cucurbits systemically. Symptoms on papaya are:

1. The first symptoms are the appearance of oily streaks on the younger leaves and the younger leaves show clearing along the veins that gives an appearance of flecks.
2. Leaves develop prominent mosaic and chlorosis on the leaf lamina, and water soaked oily streaks on the petioles and upper part of the trunk.
3. Severe symptoms often include a distortion of young leaves which also result in the development of a shoestring appearance that resembles mite damage.
4. Trees that are infected at a young stage remain stunted and will not produce an economical crop.
5. Fruit from infected trees may have bumps similar to that observed on fruit of plants with boron deficiency and often have ring spots.

6. A severe PRSV isolate from Taiwan is also known to induce systemic necrosis and wilting along with mosaic and chlorosis.



Infested field Infested leaf Infested fruit Aphids

Fig 4.6 PRSV infested ring spot diseases of Papaya

Economic impact

Papaya is grown for personal consumption by small subsistence farmers in parts of Southeast Asia, with small surpluses sold at market. PRSV is the biggest constraint to papaya production in the Philippines, Brazil, India and Nigeria.

Treatment of disease

1. Rouging, or the removal and destruction of infected plants, is a way to control the spread of PRSV.
2. Netting can also be used to prevent insect vectors aphids from spreading the virus.
3. To control aphid population insecticide Rogor or Roxion or Perfecthion 40EC or Metasystox 25 EC should be applied in formulation of 2mm/liter water.
4. It is strongly prohibited to pruning of infected plants, because virus entrance occur through the cutting parts of the plant.
5. Disease incidence can be reduced by planting a non-host crop, such as corn, around the orchard and even between rows.

Prevention

1. The most important is to establish orchards with seedlings that are not infected with PRSV. New orchards should be situated as far as feasible from infected orchards. Orchards should not be established by interplanting seedlings among trees that are infected with PRSV.
2. **Transgenic papaya** varieties that are tolerant to PRSV have been developed from breeding programs. Two transgenic varieties of papaya (**Rainbow** and **SunUp**) developed in Hawaiian of USA both of which were introduced for production in May 1998.
3. **Cross protection** is similar in practice to viral vaccinations in humans. A mild strain of PRSV is introduced into the host plant, which then develops resistance to virulent strains of the virus.
4. Prevention through quarantine and geographic displacement of cropland is common and has occurred in Hawaii, the Philippines and Brazil.

2. Hepatitis

The word hepatitis comes from the ancient Greek word *hepar* meaning 'liver', and the Latin *itis* meaning inflammation. **Hepatitis means injury to the liver with inflammation of the liver cells.** Hepatitis can be divided into two subgroups according to its duration:

(a) **Acute hepatitis**-lasting less than six months and

(b) **Chronic hepatitis**- lasting longer than six months.

Causes

Hepatitis has a number of possible causes:

1. Infectious viral hepatitis, such as hepatitis A, hepatitis B, hepatitis C, hepatitis D and hepatitis E.
2. Autoimmune hepatitis. This is a disease in which a number of liver cells are destroyed by the patient's own immune system.
3. Inborn metabolic disorders, such as **Wilson's disease** (disorder of the body's copper metabolism) and **haemochromatosis** (disorder of the body's iron metabolism).
4. Severe bacterial and amoebic infections.
5. Medicines, e.g. paracetamol poisoning and halothane (an anesthetic).
6. Toxins: alcohol and fungal toxins.

Viral hepatitis

Viral hepatitis is liver inflammation due to a viral infection. It may present in acute or chronic forms. The most common causes of viral hepatitis are the five unrelated hepatotropic viruses- *hepatitis A virus* (HAV), *hepatitis B virus* (HBV), *hepatitis C virus* (HCV), *hepatitis D virus* (HDV), and *hepatitis E virus* (HEV). In addition to the nominal hepatitis viruses, other viruses that can also cause liver inflammation include *cytomegalovirus*, *Epstein–Barr virus*, and *yellow fever*.

There is the opportunity to prevent or treat the most common types. Hepatitis A and hepatitis B can be prevented by vaccination. Effective treatments for hepatitis C are available but expensive. In 2013 about 1.5 million people died from viral hepatitis. Most deaths are due to hepatitis B and hepatitis C. East Asia is the region of the world most affected.

Characteristics of Hepatitis viruses

	HAV	HBV	HCV	HDV	HEV
Transmission	Transmitted through contaminated water or food.	Transmitted through infective blood, semen, and other body fluids.	Mostly transmitted through infective blood.	Infections occur only in those who are infected with HBV.	Transmitted through contaminated water or food.
Classification	*Picornavirus*	*Orthohepadnavirus*	*Hepacivirus*	*Deltavirus*	*Hepevirus*
Genome	+ssRNA	dsDNA-RT	+ssRNA	−ssRNA	+ssRNA
Incubation period	20–40 days	45–160 days	15–150 days	30–60 days	15–60 days
Vaccine	10 year protection	3 injections, lifetime protection	None available	None available	Investigational

Symptoms

The symptoms of acute hepatitis vary considerably from person to person. Some patients have no symptoms at all, and in most cases, children only show mild symptoms. The following are the some symptoms of hepatitis:

1. Tiredness, general malaise, slight fever.
2. Nausea, poor appetite, changes in taste perception.
3. Pressure or pain below the right ribs caused by an enlarged liver.

4. Aching muscles and joints, headache, skin rash.

5. The **jaundiced phase:** yellowing of sclera, skin and mucous membranes, dark urine, light-coloured stools, around this time, the other symptoms subside.

About one fifth of the patients with chronic hepatitis B and C are at risk of developing cirrhosis or cancer of the liver can. **Cirrhosis** can also be caused by other types of chronic hepatitis.

Diagnosis

Acute liver infection is usually suspected when patients have symptoms such as **jaundice** and **fatigue.** Blood tests will help determine the cause and severity of the hepatitis. **Surface antigen** (HBsAg) test and **HCV antibody** (anti-HCV) test recommended for hepatitis B and hepatitis C, respectively.Further information may be obtained from **ultrasound** and other types of liver scans. In certain situations a **liver biopsy** may be recommended.

Treatments

The vast majority of patients with hepatitis A will recover spontaneously. A vaccine can prevent hepatitis A. The common brands of vaccine available are *Havrix, Avaxim, BIOVAC-A* etc.

A patient with hepatitis B needs to rest. He will require a diet that is high in protein and carbohydrate - this is to repair damaged liver cells, as well as to protect the liver. If this is not enough, the doctor may prescribe interferon. A vaccine can prevents hepatitis B. The common brands of vaccine available are *Recombivax HB*, *Engerix-B* , *Elovac B* , *Genevac B*, *Shanvac B*, etc. These vaccines are given by the intramuscular route.

A patient with hepatitis C will be prescribed **pegylated interferon** and **ribavirin.** There is no effective treatment for either hepatitis D or E.

If the patient has non-viral hepatitis, the doctor needs to remove the harmful substance. It will be flushed out of the stomach by hyperventilation or induced vomiting. Patients with drug-induced hepatitis may be prescribed corticosteroids.

Prevention

1. Wash your hands with soap after going to the toilet.
2. Only consume food that has just been cooked.
3. Only drink commercially bottled water, or boiled water if you unsure of local sanitation.
4. Only eat fruits that you can peel if you are somewhere where sanitation is unreliable.
5. Only eat raw vegetables if you are sure they have been cleaned/disinfected thoroughly.
6. Get a vaccine for hepatitis A if you travel to places where hepatitis may be endemic.
7. Tell the partner if you are a carrier or try to find out whether he/she is a carrier.
8. Only use clean syringes that have not been used by anyone else.
9. Do not share toothbrushes, razors, or manicure instruments.
10. Only allow well sterilized skin perforating equipment (tattoo, acupuncture, etc.)

3. Dengue

Dengue (pronounced as *Dengee*) fever also known as **backbone fever or dandy fever** is an contagious tropical disease caused by the dengue virus. The word dengue is a Spanish word originated from the East African Swahili word *dinga* which describe the gait of a person suffering the bone pain of dengue fever. Each year, an estimated 100 million cases of dengue fever occur

worldwide. Most of these are in tropical areas of the world, with the greatest risk occurring in the Indian subcontinent, Southeast Asia, Southern China, Taiwan, The Pacific Islands, The Caribbean, Mexico, Africa, Central and South America.

Dengue virus

Dengue fever virus (DENV) is an RNA virus of the family *Flavi viridae*, genus *Flavi virus*. There are four strains of the virus, which are called serotypes, and these are referred to as DENV-1, DENV-2, DENV-3 and DENV-4.

Transmission

Dengue virus is primarily transmitted by *Aedes* mosquitoes, particularly *A. aegypti*. They typically bite during the day, particularly in the early morning and in the evening. Other *Aedes* species that transmit the disease includes *A. albopictus*, *A. polynesiensis* and *A. scutellaris*. Humans are the primary host of the virus, but it also circulates in nonhuman primates.

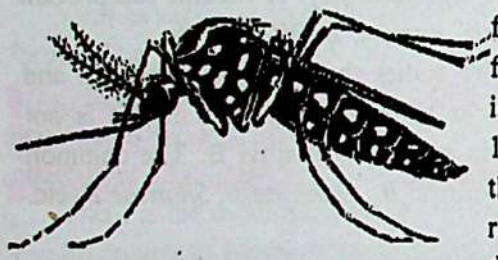


Fig 4.7 *Aedes aegypti*

An infection can be acquired via a single bite. A female *Aedes aegypti* mosquito that takes a blood meal from a person infected with dengue fever becomes itself infected with the virus in the cells lining its gut. About 8–10 days later, the virus spreads to other tissues including the mosquito's salivary glands and is subsequently released into its saliva. The virus seems to have no detrimental effect on the mosquito, which remains infected for life.

Dengue can also be transmitted via infected blood products and through organ donation. In countries such as Singapore, where dengue is endemic, the risk is estimated to be between 1.6 and 6 per 10,000 transfusions. Vertical transmission (from mother to child) during pregnancy or at birth has been reported.

Signs and symptoms

Dengue can affect anyone but tends to be more severe in people with compromised immune systems. Because it is caused by one of four serotypes of virus, it is possible to get dengue fever multiple times. However, an attack of dengue produces immunity for a lifetime to that particular serotype to which the patient was exposed.

1. Dengue starts with chills, headache, pain upon moving the eyes, and low backache.
2. Painful aching in the legs and joints occurs during the first hours of illness.
3. The temperature rises quickly as high as 104°F (40°C), with relatively low heart rate (bradycardia) and low blood pressure (hypotension).
4. The eyes become reddened. A flushing or pale pink rash comes over the face and then disappears.
5. The glands (lymph nodes) in the neck and groin are often swollen.
6. Fever and other signs of dengue last for two to four days, followed by a rapid drop in body temperature (defervescence) with profuse sweating.
7. This precedes a period with normal temperature and a sense of well-being that lasts about a day.

8. A second rapid rise in temperature follows.

9. A characteristic **rash appears** along with the fever and spreads from the extremities to cover the entire body except the face.

10 The palms and soles may be bright red and swollen.

Dengue hemorrhagic

Sometimes symptoms are mild and can be mistaken for those of the flu or another viral infection. However, serious problems can develop. These include dengue hemorrhagic fever, a rare complication characterized by high fever, damage to lymph and blood vessels, bleeding from the nose and gums, enlargement of the liver, and failure of the circulatory system. The symptoms may progress to massive bleeding, shock, and death. This is called **dengue shock syndrome** (DSS). People with weakened immune systems as well as those with a second or subsequent dengue infection are believed to be at greater risk for developing dengue hemorrhagic fever.

Dengue Treatment

Because dengue fever is caused by a virus, there is no specific medicine or antibiotic to treat it. For typical dengue, the treatment is purely concerned with relief of the symptoms. Rest and fluid intake for adequate hydration is important. Aspirin and nonsteroidal anti-inflammatory drugs should only be taken under a doctor's supervision because of the possibility of worsening bleeding complications. Acetaminophen (Tylenol) and codeine may be given for severe headache and for joint and muscle pain (myalgia).

Preventing dengue fever

There is no vaccine to prevent dengue fever. The best way to prevent the disease is to prevent bites by infected mosquitoes. This involves protecting yourself and making efforts to keep the mosquito population down. To reduce the mosquito population, get rid of places where mosquitoes can breed. These include old tires, cans, or flower pots that collect rain. Regularly change the water in outdoor bird baths and pets' water dishes. If someone in your home gets dengue fever, be especially vigilant about efforts to protect yourself and other family members from mosquitoes. Mosquitoes that bite the infected family member could spread the infection to others in your home.

To protect yourself:

- Stay away from heavily populated residential areas, if possible.
- Use mosquito repellents, even indoors.
- When outdoors, wear long-sleeved shirts and long pants tucked into socks.
- When indoors, use air conditioning if available.
- Make sure window and door screens are secure and free of holes. If sleeping areas are not screened or air conditioned, use mosquito nets.

4.5 Bacteria

Bacteria (singular: *bacterium*= staff cone=rod) are microscopic organisms whose single cells have neither a membrane-enclosed nucleus nor other membrane-enclosed organelles like mitochondria and chloroplasts etc.

Bacteria were first observed by Dutch scientist **Antony van Leeuwenhoek** in 1675. He called them '***animalcules***' and published his observations in a series of letters to the Royal Society. The name 'bacterium' was introduced much later, by German scientist **Christian Gottfried Ehrenberg**

in 1829. **Se dillot** in 1878 described them as microbes. French biologist **Louis Pasteur**, in 1869 postulated bacteria related **germ theory of disease.** German physicians **Robert Koch** (1843-1910) had proved by many experiments that many disease caused by bacteria. He discovered the bacterium *Mycobacterium tuberculosis* responsible for tuberculosis disease and for that he received the Nobel Prize in Physiology or Medicine in 1905.

Facts of Bacteria

➢ Bacteria were among the first life forms to appear on earth, and are present in most habitats on the planet.

➢ Bacteria inhabit soil, water, acidic hot springs, radioactive waste, and the deep portions of earth's crust. Bacteria also live in plants, animals, and have flourished in manned space vehicles.

➢ There are typically 40 million bacterial cells in a gram of soil and a million bacterial cells in a milliliter of fresh water.

➢ The human mouth is home to more than 500 species of bacteria.

➢ There are approximately five nonillion (5×10^{30}) bacteria on earth, forming a biomass that exceeds that of all plants and animals.

➢ Bacteria have been found that can live in temperatures above the boiling point (100°C) and in cold (-17°C) that would freeze our blood.

➢ They eat everything from sugar and starch to sunlight, sulfur and iron.

➢ Humans carry more bacterial cells than human ones. They reside on the surface and in deep layers of skin, in the saliva and oral mucosa, in the conjunctiva, and in the gastrointestinal tracts.

➢ Most of the microbes associated with humans appear to be not harmful at all, but rather assist in maintaining processes necessary for a healthy body.

➢ Many bacteria are the causal agents of many diseases in plant, animals and human.

➢ *Mycobacterium tuberculosis* bacterium is the most dangerous bacteria that killed 2 million people worldwide per year. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacterium that responsible for the death of people more than that of killed by AIDS.

Bacteriology is a branch of microbiology dealing with the identification, study, and cultivation of bacteria and with their applications in medicine, agriculture, industry, and biotechnology. **Robert Koch** designated as the father of Bacteriology.

Characteristics of bacteria

1. Bacteria are very small (0.5-2mm), simple and microscopic prokaryotic organisms.
2. They are prokaryotic i.e. no membrane-enclosed nucleus.
3. They are solitary or colonial free living or parasitic or saprozoic or symbiotic organisms.
4. They have a rigid cell wall made of peptidoglycan, resistance to environmental change.
5. They have no cellular organelles except a 70S ribosome.
6. They have a single chromosome of a closed circle of double-stranded DNA with no associated histons.
7. Some have flagella which made of a single filament of the protein flagellin.
8. Their plasma membrane contains no cholesterol or other steroids.
9. Bacteria are more susceptible to phage viruses.
10. No mitosis or meiosis; mostly asexual reproduction, if any sexual reproduction very different from that of eukaryotes.

11. Many bacteria form a single spore when their food supply runs low. Spores are so resistant to adverse conditions of dryness and temperature that they may remain viable even after 50 years of dormancy.

Classification of Bacteria

A. Based on the cell shape bacteria are classified in following ways:

1. Coccus: Coccus (plural cocci) can be used to describe any bacterium that has a spherical shape. They may be any of following six types:

(i) Monococcus: A form of coccus consisting of single cells. e.g., *Micrococcus denitrificans.*

(ii) Diplococcus: A diplococcus is a round bacterium that typically occurs in the form of two joined coccus cells. e.g., *Diplococcus pneumoniae.*

(iii) Tetracoccus: A spherical bacterium occurring in square groups of four cocci bacteria. e.g., *Gafkya tetragena.*

(iv) Streptococcus: Streptococcus is a genus of spherical coccus bacteria where cellular division occurs along a single axis and thus they grow in chains. e.g., *Streptococcus β hacmolyticus.*

(v) Staphylococcus: *Staphylococcus* is a type of non-moving small round shaped or non-motile cocci bacteria. It is found in grape-like clusters. This is why it is called staphylococcus. e.g., *Staphylococcus aureus.*

(vi) Sarcina: *Sarcina* is a genus of cocci bacteria which have a cuboidal cell arrangement. e.g., *Sarcina luta.*

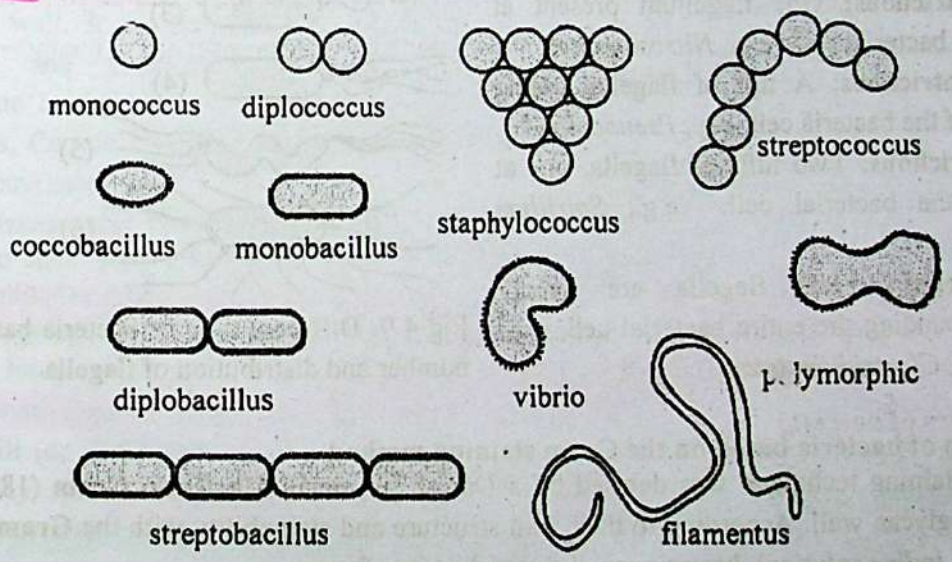


Fig 4.8. Different types (shapes) of bacteria

2. Bacillus: Bacillus is any of a group of rod-shaped, aerobic or anaerobic bacteria widely found in soil and water. The term *bacillus* has been applied in a general sense to all cylindrical or rod like bacteria. These are of four types:

(i) Monobacillus: A form of bacillus consisting of single cells. e.g., *Escherichia coli*

(ii) Diplobacillus: A diplobacillus is a rod-shaped bacterium that typically occurs in the form of two joined bacillus cells. e.g., *Diplobacillus lacunata*

(iii) Streptobacillus: Streptobacillus is a genus of rod-like bacillus bacteria where cellular division occurs along a single axis and thus they grow in chains. e.g., *Streptobacillus moniliformis.*

(iv) Palisade bacillus: They are arranged in the form of a stack. e.g., *Lampropedia sp.*

3. Coccobacillus: A coccobacillus is a type of bacterium with a shape that is intermediate between cocci and bacilli. e.g., *Gardnerella vaginalis*.

4. Spirillium: These bacteria are spirally coiled like a corkscrew. e.g., *Spirillium minus.*

5. Comma or Vibrio: These are elongated, C shaped or comma shaped bacteria. e.g., *Vibrio cholerae.*

6. Polymorphic: Polymorphic bacteria, like the *Mycoplasma pneumoniae*, can assume a number of different shapes, even within the same culture.

7. Stellate: These are star-shaped bacteria. e.g., *Stelle sp.*

8. Square: These are square shaped bacteria. e.g., *Haloquadratum walsbyi.*

9. Filamentus: These are thread shaped bacteria. e.g., *Candidatus savagella.*

B. Classification of bacteria on the basis of number of flagella

The number and distribution of flagella on bacterial surface is called **flagellation.** On this basis bacteria are of following types-

1. Atrichous: These bacteria have no flagella. e.g., *Lactobacillus, Pasteurella.*

2. Monotrichous: Single flagellum present at one end of the bacterial cell. e.g., *Vibrio cholera.*

3. Amphitrichous: One flagellum present at each end of the bacterial cell. e.g., *Nitrosomonas.*

4. Cephalotrichous: A tuft of flagella arising from one end of the bacteria cell. e.g., *Pseudomonas.*

5. Lophotrichous: Two tufts of flagella, one at each end of the bacterial cell. e.g., *Spirillum volutans.*

6. Peritrichous: The flagella are evenly distributed surrounding the entire bacterial cell. e.g., *Escherichia coli, Clostridium tetani.*

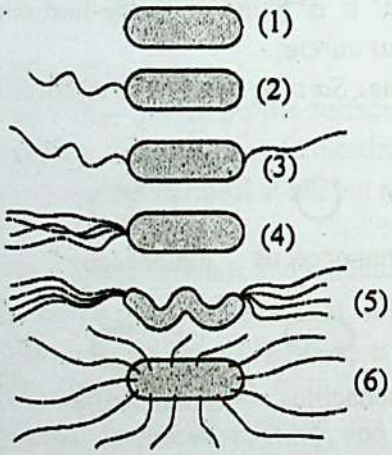


Fig 4.9. Different type of bacteria based on number and distribution of flagella.

C. Classification of bacteria based on the Gram staining method

The Gram staining technique was devised by a Danish physician **Christian Gram** (1884) to stain the peptidoglycan wall. According to their wall structure and stainability with the **Gram stain** (*Crystal violet + iodine solution*), bacteria are distinguished into 2 types:

1. Gram positive bacteria (G^+): Gram positive bacteria retain the **Gram stain** and have uniformly thick peptidoglycan wall (10-80nm) with less lipid content and more acidic protoplasm. e.g., *Bacillus subtilis, Staphylococcus aureus, Streptococcus pneumonia etc.*

2. Gram negative bacteria (G^-): The Gram negative bacteria don't retain (loss) the Gram stain, have double layered cell wall (7.5-12 nm), with thin inner peptidoglycan wall and high lipid content but they take up the red colour of the counter stain like saffranin. e.g., *Salmonella bongori, Escherichia coli, Neisseria meningitidis, Vibrio cholerae* etc.

Structure of a typical Bacterium

Bacterial cells are prokaryotic type. A typical bacterium comprises of the the following parts:

1. Capsule and slime layers: A lose gelatinous sheath called slime layer is usually deposited around the bacterial cell wall. It is usually composed of polysaccharides. In some bacteria, the slime layer becomes thick due to the presence of some nitrogen compounds and forms a capsule. A capsule is particularly common in the parasitic forms. This protects the cell in adverse conditions.

2. Cell wall: It is a 10-25 micron wide and elastic outer tough covering of around the cell, which provides specific shape and protection to the bacterium. It prevents the cell from swelling and bursting due to osmotic changes. It is composed of a polysaccharide called **murein** or **peptidoglycan**. It consists of polysaccharide cross linked with short amino acid chains. The cell wall of gram positive bacteria is much thicker and contains fewer lipids compared to that of Gram negative bacteria. The cell wall can be dissolved by the enzyme **lysozyme**.

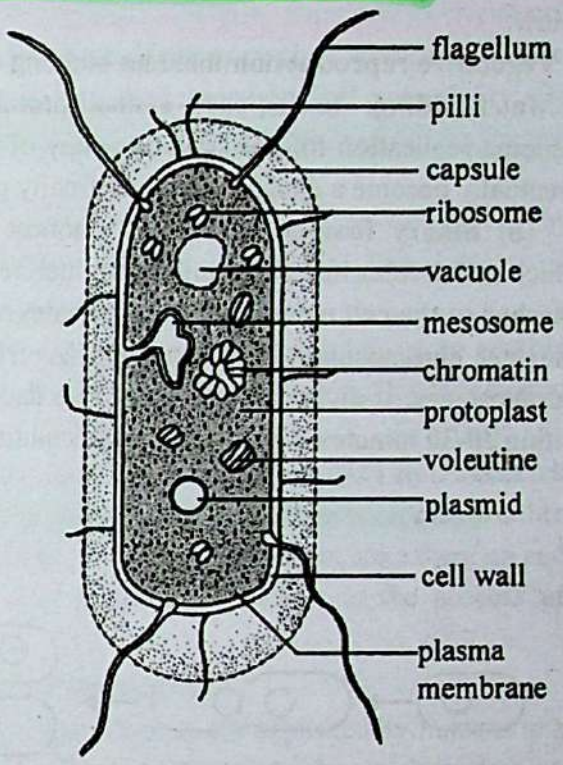


Fig 4.10 Structure of a typical bacterium

3. Plasma membrane: It is thin, elastic, selectively permeable membrane found internal to the cell wall. It is composed of phospholipids, proteins and polysaccharides. The plasma membrane is the site of most of the metabolic pathways. Certain structures are found associated with plasma membrane.

4. Mesosomes: These are invaginations formed by the plasma membrane. They are spherical or elongated structures which bring about a functional compartmentalisation of the cell. They have significant roles in cell division and replication. They are more common in Gram positive bacteria.

5. Flagella: These are long, slender thread-like structures, which help in locomotion. A bacterial cell may have one to many flagella. The flagella are entirely composed of a protein called **flagellin**. Bacteria with flagella are known as **trichous** and without flagella are called **atrichous**.

6. Pili (or Fimbriae): These are extremely minute hair-like structures found mostly in male cells. They are composed of protein subunits called **pilin**. They take part in the formation of conjugation tube. They are therefore called **sex pili**.

7. Cytoplasm: It is a semi-fluid ground substance enclosed by the plasma membrane. It appears granular due to the presence of large number (as many as 20,000) ribosome. The ribosome may occurs singly or in clusters called **polyribosomes.** Membrane-bound cell organelles like mitochondria, lysosomes, Golgi complex, vacuoles and endoplasmic reticulum are absent.

8. Nucleoid (or Bacterial Chromosome): Since the bacterial cell is prokaryotic, a true nucleus is absent. The nuclear material is represented by DNA which is not associated with histones. It is identified as a **nucleoid** or bacterial chromosome. It is a circular ring. It is attached at a point to the plasma membrane.

9. Plasmids: Apart from the nucleoid, certain bacterial cells contain additional rings of DNA called **plasmids.** The plasmid DNA replicates independently. It has certain genes like fertility factor (F-factor), resistance factor (R-factor), nitrogen fixing genes (Nif-genes). Some plasmids may temporarily become associated with nucleoid DNA and are known as **episomes.**

Reproduction of Bacteria

Bacteria reproduce by vegetative, asexual and sexual methods. These methods are briefly described below:

1. Vegetative reproduction includes budding and binary fission

(a) Budding: In this case, a small protuberance, called bud, develops at one end of the cell. Genome replication follows, and one copy of the genome gets into the bud. Then the bud enlarges, eventually become a daughter cell and finally gets separated from the parent cell.

(b) Binary fission: It is the commonest type of reproduction under favourable conditions in which cell divides into two similar daughter cells. During the process, the bacterial chromosomes get attached to the cell membrane and replicates to the bacterial chromosomes. As the cell enlarges the daughter chromosomes gets separated. A cross wall is formed between the separating daughter chromosomes. It divides the cell into two daughter cells. The daughter cells soon grow to maturity within 20-30 minutes. Under favourable conditions many bacteria divide once in 20-30 minutes.

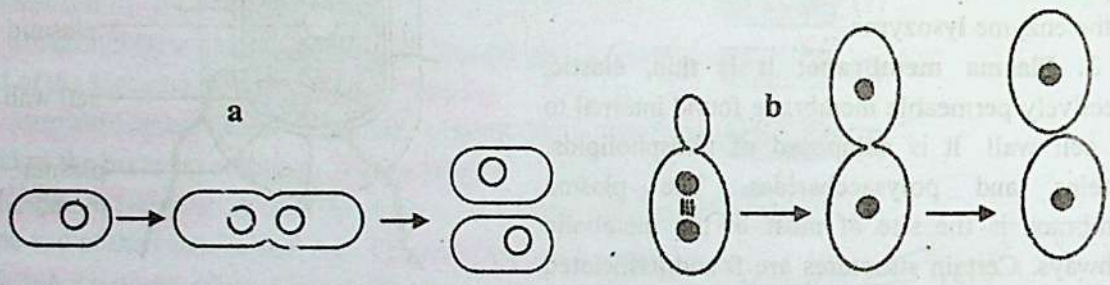


Fig 4.11 Vegetative reproduction of Bacteria (a) Binary fission (b) Budding

2. Asexual reproduction: In adverse condition bacteria reproduce asexually by following three methods:

(a) By formation of conidia: A certain bacteria like *Streptomycin* cuts off tiny, oval or rounded, non motile structures called conidia (pl- Conidia, sing- Conidium) in chains. These are borne at the tips of special aerial branches called conidiophores. Each conidium under suitable conditions germinates into new bacterium.

(b) By formation of gonidia: Mostly during unfavourable conditions, bacterial protoplasm undergoes compartmentalization and subsequent fragmentation, forming minute bodies called **gonidia.** Under favourable conditions, each gonidium grows to a new bacterium.

(c) By formation of endospores: Endospores are resting spores formed in some Gram positive bacteria (*Bacillus* and *Clostridium*) during unfavourable conditions. They are formed within the cells. During this process a part of the protoplast becomes concentrated around the chromosome. A hard resistant wall is secreted around it. The rest of the bacterial cell degenerates. Endospores are very resistant to extreme physical conditions and chemicals. During favourable conditions the spore wall gets ruptured and the protoplasmic mass gives rise to a new bacterium.

3. Sexual reproduction: As stated earlier in the middle of twentieth century, bacteria do not reproduce sexually like eukaryotic organisms. In 1946 **Joshua Lederberg** and **Edward L. Tatum** discovered a type sexual reproduction in *Escherichia coli* bacteria where there is no gamete formation, but sexual reproduction occurs in the form of **genetic recombination.** In 1958, **Lederberg and Tatum** received the Nobel Prize in Medicine for their discovery, shared with **George W. Beadle.**

Genetic recombination refers to the transfer of DNA or genetic material from one bacterial cell (donor) to another cell (recipient). It results in new combinations of genes on the chromosome. After reproduce several times through binary fissions bacteria loss their physiological potentiality. By genetic recombination bacteria rejuvenilized them. In bacteria genetic recombination can occurs three ways:

(a) Conjugation: Conjugation is the transfer of genetic material between bacterial cells by direct cell-to-cell contact or by a bridge-like connection between two cells. This process is discovered in 1946 by **Joshua Lederberg** and **Edward Tatum.**

(b) Transduction: This method involves the transfer of DNA from one bacterium to another with the use of a bacteriophage (phage virus). In 1952 **Zinder and Lenderberg** have discovered transduction in *Salmonella* bacteria.

(c) Transformation: The transformation process was first demonstrated in 1928 by **Frederick Griffith.** After death or cell lyses, some bacteria release their DNA into the environment. Other bacteria, generally of the same species, can come into contact with these fragments, take them up and incorporate them into their DNA by recombination. This method of transfer is the process of **transformation.**

Economic Importance of Bacteria

The economic importance of bacteria derives from the fact that bacteria are exploited by humans in a number of beneficial ways. Despite the fact that some bacteria play harmful roles, such as causing disease and spoiling food. The economic importance of bacteria includes both their useful and harmful aspects.

Useful aspects of bacteria

1. In agriculture

(i) Decay of dead plants and animals: Some bacteria attack dead bodies of plants and animals and convert their complex compounds into simpler substances, as carbon dioxide (CO_2) water (H_2O), nitrate (NO_3), sulphate (SO_4), etc. e.g. *Pseudomonas* bacteria.

(ii) Nitrification: *Rhizobium* bacteria, living in root nodules of leguminous plant symbiotically, helps in fixing atmospheric nitrogen. Similarly, *Nitrosomanas* and *Nitrococcus* convert ammonium salt to nitrites. Nitrites are further changed to nitrates by *Nitrobacter* and *Nitrocystis.* It enables plants to uptake nitrogen.

(iii) Preparation of ensilage: Ensilage is preserved cattle fodder prepared by packing fresh chopped fodder sprinkled with molasses. Fermentation activity of bacteria produces lactic acid that acts as preservative in ensilage.

(iv) Production of biofuel: Bacteria, while converting animal dung and other organic wastes to manure, help in production of biofuel that is a must in global gas plant.

(v) Pest control: Bacteria can also be used in the place of pesticides in the biological pest control. This commonly uses *Bacillus thuringiensis* (also called *Bt*), a Gram-positive, soil dwelling bacterium. This bacterium is used as a Lepidopteran-specific insecticide under trade names such as Dipel and Thuricide.

2. In Industry

(i) Dairy industry: Bacteria such as *Streptococcus lactis* convert milk sugar lactose into lactic acid that coagulates casein (milk protein). Then, milk is converted into curd, yoghurt, cheese etc needed for the industry.

(ii) Production of organic compounds: Fermentation action of various bacteria produces organic compounds like lactic acid (by *Lactobacillus*), acetic acid (by *Acetobacter aceti*) etc.

(iii) Fiber ratting: The action of some bacteria like *Clostridium butyricum, Pseudomonas* etc. help in fiber ratting i.e. separation of stem and leaf fiber of plants from other softer tissue.

(iv) Curing: The leaves of tea and tobacco, beans of coffee and coca are cured off their bitterness with the help of action of certain bacteria such as *Bacillus megatherium.*

(v) Production of antibiotics: Number of anti bacterial and anti fungal antibiotics such as Hamycin, Polymyxin, Trichomycin etc. are obtained from mycelia bacteria (like *Streptomyces*). Similarly, *Bacillus* is used for production of antibiotics such as Bacitracin, Gramicidin etc.

(vi) Production of vitamins: Different kinds of vitamins are produced from bacteria like Riboflavin from *Clostridium butylicum*, Vitamin B_{12} from *Bacillus megatherium* and Vitamin K and B-complex from *Escherichia coli.*

(vii) Production of enzymes: *Bacillus subtilis, Clostridium histolyticum, Trichoderma konigi* etc. bacteria are used in commercial production of enzymes.

(viii) Vinegar industry: Vinegar is manufactured from sugar solution in the presence of *Acetobacter aceti.*

(ix) Alcohol and Acetone: *Clostridium acetobutylicum* takes part in the manufacture of butyl alcohol and acetone.

3. In Genetic engineering

Using biotechnology techniques, or bio medical technology bacteria such as *Escherichia coli, Agrobacterium tumefaciens* etc. can also be bioengineered for the production of therapeutic proteins, such as vaccine, interferon, insulin, growth factors or antibodies.

4. In cellulose digestion

Some bacteria living in the gut of cattle, horses and other herbivores secrete cellulase, an enzyme that helps in the digestion of the cellulose contents of plant cell walls. Cellulose is the major source of energy for these animals. Generally plant cells contain cellulose. The bacteria present in the stomach of cattle will help in the digestion of cellulose.

5. Disposal of sewage

Bacteria help in disposal of sewage by decomposing it and thus, help in environmental sanitation. *Pseudomonus, Nocardia, Mycobacterium* etc. bacteria remove petroleum from water.

6. Probiotic bacteria

Probiotic bacteria are bacteria that are good for our health, especially our digestive system. Probiotics are often called "good" or "helpful" bacteria because they help keep our gut healthy. It has been estimated that there are about 500 species of probiotic bacteria found inhabiting human colon.

Harmful aspects of bacteria

Though bacteria plays irnportant role in agriculture, industries and natural sanitation etc, it has the following harmful effects:

1. **Food spoiling:** Saprophytic bacteria always not only help in decomposition of dead matters, but they also cause the rotting of vegetables, fruits, meat, bread etc. Food spoiling bacteria are *Clostridium, Staphylococcus, Pseudomonas, Acinetobacter, Bacillus* etc.
2. **Food poisoning:** Bacteria like *Staphylococcus aureus* cause food poisoning and cause people diarrhea and vomiting. *Clostridium botulinum* bacteria poisoning the botulized food and causes **botulism** for man.
3. **Damaging of domestic articles:** *Spirochete cytophaga* deteriorates cotton, leather and wooden articles.
4. **Denitrification:** Bacteria such as *Thiobacillus* and *Microbacillus* convert nitrate of the soil to the gaseous nitrogen. This hampers plants very much.
5. **Desulphurication:** Bacteria such as *Desulfovibrio* convert soil sulphates into hydrogen sulphide.
6. **Causes of pollution:** *E. coli, Salmonella, Vibrio* etc. bacteria causes water pollution.
7. **Cause of diseases:** It is known that over 90% of human diseases and over 10% of plant diseases are caused by bacteria.

Human diseases: Cholera *(Vibrio cholerae)*, Pneumonia *(Diplococcus pneumoniae)*, Tuberculosis *(Mycobacterium tuberculosis)*, Diphtheria *(Corynebacterium diphtheriae)*, Typhoid *(Salmonella typhosa)*, Tetanus *(Clostridium tetani)*, Gonorrhoea *(Neisseria gonorrhoeae)*, Meningitis *(Neisseria meningitidis)*, Syphilis *(Treponema pallidum)*, Ulcer *(Streptococcus sp)*, Urinary infection *(Kelbsiell sp) etc.*

Plant diseases: Potatoes scab *(Streptomyces scabies)*, Canker of Tomato (*Corynebacterium michiganese)*, Rice blight *(Xanthomonus oryzae)*, Fire blight of apple (*Erwinia amylovora)*, Crown gall of tomato *(Agrobacterium tumefaciens)*, Crown gall of rose *(Agrobacterium tumefaciens)*, Tobacco blight *(Pseudomonas tabacci)*, Bean leaf spot *(Xanthomonus malvacearum)* etc.

Animal disease: Plague of mice, Galander of goat and sheep.

4.5 Bacterial Diseases

❑ Leaf blight diseases of rice

Leaf blight of rice also known as rice **bacterial leaf blight, rice kresek disease, bacterial leaf blight of rice, kresek disease** is one of the most destructive diseases of rice in Asia especially in the irrigated and rain fed lowland ecosystems. Bacterial blight is reported to have reduced Asia's annual rice production by as much as 60%.

Causes

The causal organism is the bacterium, *Xanthomonas oryzae pv. Oryzae* (Ishiyama 1922 Swings *et al.* 1990). According to the new classification system, it has been placed in the family Xanthomonadaceae of the phylum Proteobacteria.

Transmission

- Lateritic and alluvial soil favour more bacterial blight diseases.

- Water logging condition encourages disease development.
- Excessive use of N fertilizers from tillering stage to maximum tillering stage. encourages disease development.
- Growing of the crop under shade favours disease development.
- Pruning of leaves at the time of transplanting favours disease development.

Symptoms

1. The earliest symptom of the blight phase is the appearance of dull greenish water-soaked or yellowish spots 5-10 mm in length on the leaf towards the tip or margins, leading to tip and marginal dying. The infection soon extends along one or both margins, sometimes to the leaf sheath.

2. As the disease progress, several lesions coalesce to form straw-brown large lesions or blighted portions. The inner margin of the blighted patch in contact with the adjoining green portion of the leaf is ragged or wavy.

3. Occasionally, the lesion may extend from the tip downward along the midrib itself, the leaf margins remaining green. Small droplets of bacterial ooze, pale amber in colour, are found on the affected portions.

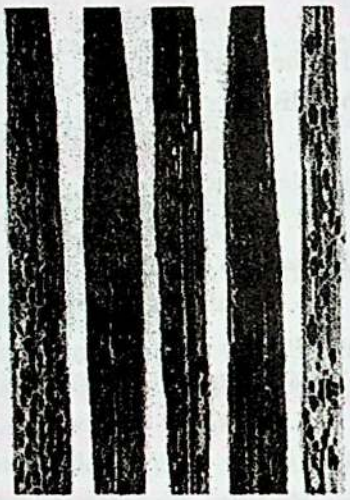


Fig 4.12 *X. oryzae* infested leaves of rice

Control methods

Practicing field sanitation such as removing weed hosts, rice straws, ratoons, and volunteer seedlings is important to avoid infection caused by this disease. Likewise, maintaining shallow water in nursery beds, providing good drainage during severe flooding, plowing under rice stubble and straw following harvest are also management practices that can be followed. Proper application of fertilizer, especially nitrogen, and proper plant spacing are recommended for the management of bacterial leaf blight.

The use of resistant varieties is the most effective and the most common management practices adopted by farmers in most growing countries in Asia. When different strains of bacteria are present, it is recommended to grow resistant varieties possessing field resistant genes. Fallow field and allow to dry thoroughly is recommended.

Seed treatment with bleaching powder (100µg/ml) and zinc sulfate (2%) reduce bacterial blight. Control of the disease with copper compounds, antibiotics and other chemicals has not proven highly effective.

❑ Cholera

Cholera is an infectious disease that causes severe watery diarrhea, which can lead to dehydration and even death if untreated. It is caused by eating food or drinking water contaminated with a bacterium called *Vibrio cholerae*. Cholera outbreaks are still a serious problem in several parts of the world, where cholera affects an estimated 3 to 5 million people and causes more than 100,000 deaths each year. The disease is most common in places with poor sanitation, crowding, war, and famine. Common locations include parts of Africa, south Asia, and Latin America.

Symptoms and signs

The symptoms and signs of cholera are a watery diarrhea that often contains flecks of whitish material that are about the size of pieces of rice. The diarrhea is termed **rice-water stool and smells fishy.** The volume of diarrhea can be enormous; high levels of diarrheal fluid such as 250 cc per kg or about 10 to 18 liters over 24 hours for a 70 kg adult can occur. People may go on to develop one or more of the following symptoms and signs:

-Vomiting, Rapid heart rate, Loss of skin elasticity, Dry mucous membranes, Low blood pressure, Thirst, Muscle cramps, Restlessness or irritability (especially in children)

If not treated, dehydration can lead to shock and death in a matter of hours.

Causes and transmission

Cholera is caused by the bacterium *Vibrio cholerae* is usually found in food or water contaminated by feces from a person with the infection. Although there are many *V. cholerae* serotypes that can produce cholera symptoms, the O groups O_1 and O_{139}, which also produce a toxin, cause the most severe symptoms of cholera. The toxin produced by these *V. cholerae* serotypes is an **enterotoxin.** The enterotoxin causes human cells to extract water and electrolytes from the body and pump it into the intestinal lumen where the fluid and electrolytes are excreted as diarrheal fluid.

The bacteria are usually transmitted by drinking contaminated water, but the bacteria can also be ingested in contaminated food, especially seafood such as raw oysters. Common sources of transmissions include:

- Municipal water supplies.
- Ice made from municipal water.
- Foods and drinks sold by street vendors.
- Vegetables grown with water containing human wastes.
- Raw or undercooked fish and seafood caught in waters polluted with sewage.

Treatment

Hydration is the mainstay of treatment for cholera. Depending on how severe the diarrhea is, treatment will consist of oral or intravenous solutions to replace lost fluids. Oral rehydration salts (ORS) fluids are available in prepackaged containers, commercially available worldwide, and contain glucose and electrolytes.

In general, antibiotics are reserved for more severe cholera infections; they function to reduce fluid rehydration volumes and may speed recovery. Severe infections have been effectively treated with tetracycline, doxycycline, furazolidon, erythromycin or ciprofloxacin in conjunction with IV hydration.

In addition, there are two oral cholera vaccines available, Dukoral (manufactured by SBL Vaccines) which is World Health Organization (WHO) prequalified and licensed in over 60 countries, and ShanChol (manufactured by Shantha Biotec in India), which is licensed in India and is pending WHO prequalification.

Prevention

Developed countries have an almost zero incidence of cholera because they have widespread water-treatment plants, food-preparation facilities that usually practice sanitary protocols, and most people have access to toilets and hand-washing facilities. We can protect us and our family by using

only water that has been boiled, water that has been chemically disinfected or bottled water. Be sure to use the bottled, boiled, or chemically disinfected water for the following purposes:

-Drinking, preparing food or drinks, making ice, brushing your teeth, washing your face and hands, washing dishes and utensils that you use to eat or prepare food, washing fruits and vegetables.

Research is ongoing

There are over 30 universities researching this disease (cholera's epidemiology, pathology, immunology, vaccine production, and other problems) currently worldwide.

4.5 Experiment: Identification of bacteria from sour Card/Yogurts.

Theory: It is possible to identify *Lactobacillus* and *Streptococcus* bacteria from sour card /yogurts through the Gram-staining method.

Requirements

A. Equipment: Clean and dry microscope slide, Inoculating loop, Blotting paper, Bunsen burner, Test tub, Water, Light microscope.

B. Reagents: 1% Gram violet stain, 1.5% Iodine solution, Alcohol-acetone mixture, 1% safranin solution, Suspension of sour card, Emersion oil.

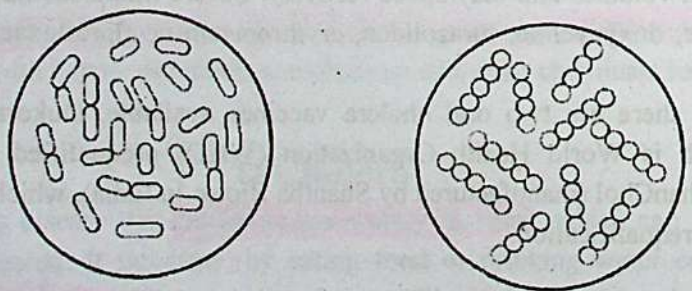
Methods

A. Prepare a Slide Smear

1. Transfer a drop of the suspended culture to be examined on a slide with an inoculation loop.
2. Spread the culture with an inoculation loop to an even thin film over a circle of 1.5 cm in diameter, approximately the size of a dime.
3. Allow the culture to air dry and then heat fix in the usual manner. Heat fixation is performed by the rapid passage of the air-dried smear two or three times over the flame of the Bunsen burner.

Gram Staining

4. Add 1% crystal violet stain over the fixed culture. Let stand for 10 to 60 seconds.
5. Pour off the stain and gently rinse the excess stain with a stream of water from a plastic water bottle.



Lactobacillus *Streptococcus*

Fig 4. 13. Card bacteria

6. Add the iodine solution on the smear, enough to cover the fixed culture. Let stand for 10 to 60 seconds.

7. Pour off the iodine solution and rinse the slide with running water. Shake off the excess water from the surface.

8. Decolorize with ethyl alcohol. Caution: Do not over-decolorize. Add reagent drop by drop until alcohol run almost clear, showing only blue tinge. Rinse it off with water after 5 seconds.

9. Counterstain with 1% safranin or 0.2% eosin for 30 seconds. Wash off the solution with water.

10. Blot with bibulous paper to remove the excess water and examine under oil immersion.

Observation

- Bacteria appear purple as stained and spherical shaped are, *Streptococcus*.
- Bacteria appear purple as stained and rod shaped are, *Lactobacillus*.

Differences between Virus and Bacteria

Virus	Bacteria
1. Viruses are acellular particles or without cellular organization.	1. Bacteria are prokaryotic cells that display all of the characteristics of living organisms.
2. Size ~20 to 400nm in diameter, only visible under electron microscope.	2. Size ~200 to 5000 nm in diameter, visible under light microscope.
3. Strict intracellular infectous agents, always requires a living host.	3. Can live inside or outside host. Living in a variety of environments.
4. Virus can be crystallized preserving their living properties.	4. Bacteria are living and cannot be crystallized.
5. Antibiotics can kill bacteria	5. Antibiotics cannot kill viruses.
6. Genetic material can be DNA or RNA, never both together.	6. Genetic material is always DNA.
7. Bacteria are harmless and some are even beneficial to humans, other bacteria are capable of causing disease.	7. Viruses are pathogens that cause a range of diseases.

4.7 Malarial Parasite

What is malaria?

Malaria is a mosquito-borne infectious disease of humans and other vertebrate animals (e.g. reptiles, birds, rat, monkey etc.) caused by the protozoan parasite of 60 species of the genus *Plasmodium* of the phylum Apicomplexa. Four species of *Plasmodium* can infect and be transmitted by humans. Commonly, in human the disease is transmitted via a bite from an infected female *Anopheles* mosquito, which introduces the organisms from its saliva into a person's circulatory system. Malaria causes symptoms that typically include fever and headache, which in severe cases can progress to coma or death. The vast majority (95%) of deaths are caused by *P. vivax*.

Epidemiology

The WHO estimates that in 2015 there were 214 million cases of global malaria resulting in 438 000 deaths. The majority of cases (65%) occur in children under 15 years old. Malaria is presently endemic in a broad band around the equator, in areas of the Americas, many parts of Asia, and much of Africa. According to the WHO, malaria is prevalent in over 100 countries. Half of the global population remains under the risk of malaria. Malaria is one of the major public health problems in

Bangladesh. Out of the total 64 districts 5 districts of Chittagong Hill Tracts are in the high endemic areas of malaria transmissions.

Historical background

Malaria may have contributed to the decline of the Roman Empire, and was so pervasive in Rome that it was known as the "Roman fever". The term malaria originates from Medieval Italian: *mala aria* means bad air. The disease was formerly called *ague* or *marsh fever* due to its association with swamps and marshland. **Torti** in 1753 first used the word '*malaria*' and **Macculoach** (1827) first of all applied this term for the marsh fever. Scientific studies on malaria made their first significant advance in 1880, when **Charles Laveran**, a French army doctor observed parasites inside the red blood cells of infected people for the first time. For this and later discoveries, he was awarded the Nobel Prize for Physiology or Medicine in 1907. In 1897 British doctor **Sir Ronald Ross**, who was working in the Presidency General Hospital in Calcutta, provided strong evidence that mosquitoes were transmitting disease to and from humans and discovered the complete life-cycle of the malaria parasite in mosquitoes. For this work, Ross received the Nobel Prize in Physiology or Medicine in 1902.

Systematic Position of malarial parasite

According to **Meglitsch** and **Schram** (1991) the classification of malarial parasite, *Plasmodium vivax* is-

Kingdom	: Protista
Subkingdom	: Protozoa
Phylum	: Apicomplexa
Class	: Sporozoa
Order	: Haemosporidia
Family	: Plasmodiidae
Genus	: *Plasmodium* Marchiafava & Celli, 1885
Species	: *Plasmodium vivax* Grassi & Feletti 1890

Malarial parasites and malarial fever in Human

Among the 60 species of the genus *Plasmodium*, only 4 species cause malaria disease of different kinds in man. These are:

Name of malaria parasite	Name of disease	Nature of fever
1. *Plasmodium vivax*	Benign tertian malaria	48 hours intermission
2. *Plasmodium ovale*	Mild tertian malaria	48 hours intermission
3. *Plasmodium falciparum*	Malignant tertian malaria	36-48 hours intermission
4. *Plasmodium malariae*	Quartan malaria	72 hours intermission

Plasmodium malariae and *Plasmodium ovale* are not found in Bangladesh.

Host of malaria parasite:

Malaria parasite has two host, man and female *Anopheles*. Man is designated as **primary host** and the *Anopheles* **secondary host** or **vector**. Worlwide there are 430 species of mosquitoes in the genus *Anopheles*, among them only 19 are responsible for malarial vector. In Bangladesh 34 species

of *Anopheles* have been identified but 8 are responsible for malarial vector. The most common are- *A. dirus, A. aconitus, A. annularis, A. philippnensis, A. sundaicus.*

■ **Only female *Anopheles* mosquitoes spead the malaria**

Only female *Anopheles* mosquitoes can spead the malaria, because they have piercing and sucking type mouth parts adapted for intake blood from the vertebrate body. The vertebrate blood is essential for the develoment of mosquitos eggs. On the other hand, male *Anopheles* mosquitoes have blunt maxillary palps, fused hypopharynx and labium in their mouthparts that unsuit the proboscis to intake blood from the vertebrate body.

■ **The female *Culex* and *Aedes* mosquitoes do not spread the malaria**

The female *Culex* and *Aedes* mosquitoes suck the blood from human body, but do not spread the malaria. They have special enzyme in their digestive juice that can able to digests the gametocytes of malarial parasite.

Life cycle of malaria parasite, *Plasmodium vivax*

Plasmodium vivax is an intracellular parasite and completes its life cycle within two host. Human is called the primary or intermediate host where the parasite reproduces by **schizogony** (within hepatic/liver cell and RBC) which is an asexual process. Female *Anopheles* mosquito is called the secondary or definitive host where the parasite reproduces by **sporogony** (within crop lumen and its wall) which a kind of sexual reproduction. The life cycle of *P. vivax* completed as in the following outline:

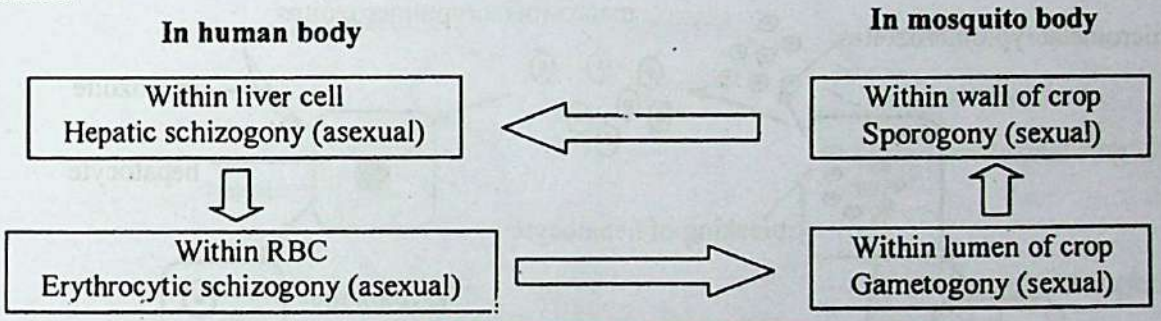


Fig 4. 14 Outline of life cycle of *Plasmodium vivax*

Schizogony or asexual cycle in human body

The asexual division of *P. vivax* parasite in human body is called **schizogony.** In human body the *P. vivax* always remain as haploid (n) condition. Schizogony occurs in hepatocytes (liver cell) and in red blood corpuscles (RBC). The schizogony of hepatocytes and RBC is called respectively as **hepatic schizogony** and **erythrocytic schizogony.**

A. Hepatic schizogony

The infective stage of *P. vivax* present in salivary glands of *Anopheles* is known as **sporozoite.** Sporozoites enter the human blood circulation via saliva of mosquito, when a female *Anopheles* that carrying sporozoite bites a healthy man during sucking blood. The sporozoites then enter the cells of the human liver within 30 minutes of invasion into blood. **Shortt** and **Garnham** (1948) described the hepatic schizogony of *P. vivax*. This includes the following consecutive stages:

1. Sporozoite: Sprozoites are very small (10-14μm length and 0.5-1 μm wide), slightly bended, spindle shaped active parasitic stage and with single haploid nucleus. They enter hepatocytes within 30 minutes of infection in human body.

2. Cryptozoite: After entering into a hepatocyte (liver cell) the sporozoite becomes round, grows in size by taking food. This stage is called cryptozoite.

3. Schizont: The nucleus of each cryptozoite divides repeatedly to form numerous small nucleus (1000-1200). This stage of multinucleated parasite is called schizont.

4. Cryptomerozoite: Inside the schizont cytoplasm is deposited around each small nucleus and form new cell. Each of the cell is called cryptomerozoites. Cryptomerozoites come out of the hepatic cell by breaking the plasma membrane of the schizont and hepatic cell and stay sinusoid of liver.

The schizogony or asexual cycle of the *P. vivax* within hepatic parenchymal cell from the sporozoite to cryptomerozoite is known as pre-erythrocytic schizogony.

5. Metacryptomerozoites: Cryptomerozoite from sinusoid invades the new hepatocyte and become large by taking food. It divides repeatedly through schizont stages and produces numerous parasites. These are called metacryptomerozoites or phanerozoites. These invade new hepatocytes and produced a large number of metachroptozoites in same way. Among metacryptomerozoites some are become small, called **micro-metacrytomerozoite** and some are become large, called **macro-metacryptomerozoites.** Macrometacrytomerozoites invade the hepatocytes but micro-metacryptomerozoites attack the RBC.

The schizogony or asexual cycle of the *P. vivax* within hepatic parenchymal cell from the cryptomerozoite to micro and macrometacryptomerozoite is known as exo-erythrocytic schizogony.

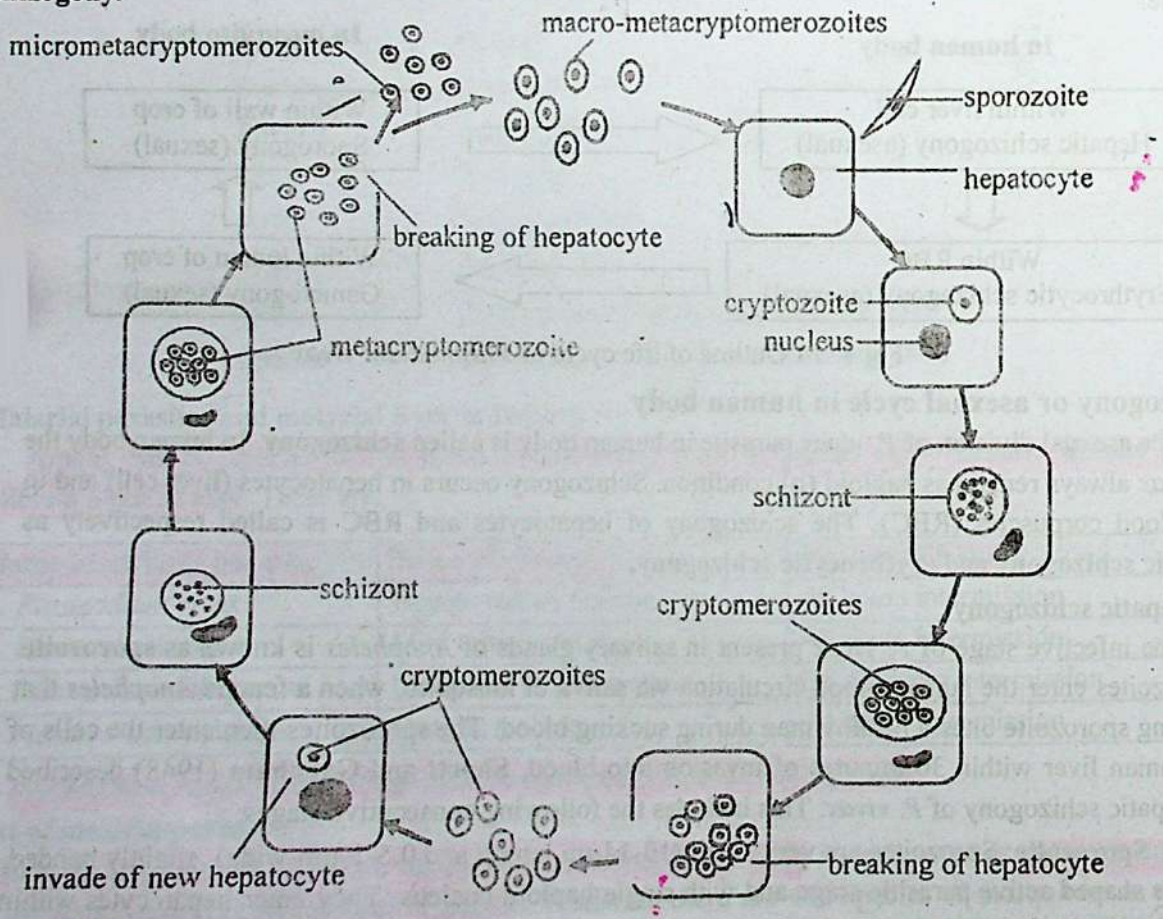


Fig 4.15 Hepatic schizogony of *P. vivax*

B. Erythrocytic schizogony

From entering of sporozoite into liver cells it takes 7-8 days to expression of the parasites in the blood. This time is called **pre-patent period.** Sometimes, after entering into the liver cell the sporozoite remain dormant and does not divide. These, after few months or years may divide producing merozoites. Such dormant sporozoite is called **hypnozoite.**

The erythrocytic schizogohy begins when a micro-metacryptomerozoite attacks the red blood cell or erythrocyte. The erythrocytic shizogony is completed by the following consecutive stages:

1. Trophozoite: Each micro-metacryptomerozoite after invading red blood cell starts eating haemoglobin become a large and round shape structure with a single nucleus. This stage of parasite is called trophozoite.

2. Signet ring: As the trophozoite grows a vacuole appears in the centre and the nucleus is pushed to one side. This stage of the parasite is clinically referred to as **signet ring stage** because it resembles a Signet Ring (round seal), with the peripheral nucleus looking like a gem of the ring.

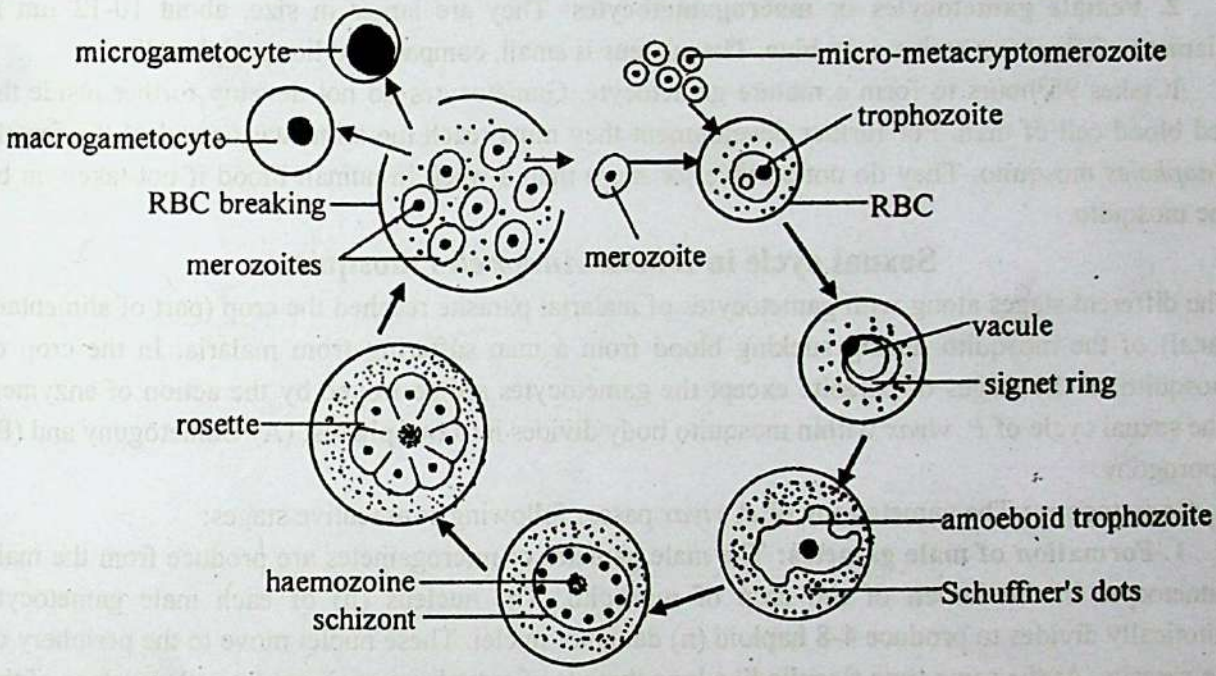


Fig 4.16 Erythrocytic schizogony of *P. vivax*

3. Amoeboid trohpozoite: The vacuole of the signet ring stage gradually diminishes due to taking more food and the parasite takes the shape of *Amoeba* with pseudopodia. Hence this stage is known as **amoeboid trophozoite.** During this period the red blood cells become larger, almost double of its original size and numerous fine characteristic granules are seen in the cytoplasm. The granules are known as **Schuffner's dots.**

4. Schizont: Due to active feeding the pseudopodia of amoeboid trophozoite diminishes gradually and the parasite becomes round in shape. During this time the nucleus divides by asexual process into 12-24 daughter nuclei. This stage of the parasite with many nuclei is called **schizont.** Simultaneously the ingested hemoglobin of the blood cell decomposed into amino acid and haematin. The amino acid is utilized by parasite and haematin is converted to toxic **haemozoine granules.** The nuclei become to lie on the periphery while the haemozoin granules accumulated at the center.

5. Merozoite: Each nucleus of the schizont is now surrounded by a little amount of cytoplasm. Thus many small uninucleated round cells are formed which are known as **merozoite.** Primarily these merozoites arranged as the petals of rose hence called the **rosette.** The mature merozoites along with haemozoin after breaking the membrane of the red blood cells set free in the blood plasma and invading again the new blood cell repeats the cycle in the same way.

It takes 48 hours by *Plasmodmm vivax* to complete an erythrocytic cycle. At the end of each cycle a large number of merozoites are released in the plasma as a result of which fever with chill and rigor is expressed in the patient.

Formation of gametocyte

Some merozoites that produced in the erythrocytic schizogony cycle develop into gamatocytes inside the red blood cells. The gamatocytes are of two type, *viz.*,-

1. Male gametocytes or **microgametocytes:** They are smaller in size, about 9-10 µm in diameter. The cytoplasm stain light blue, the nucleus is large, laterally placed and diffused.

2. Female gametocytes or **macrogametocytes**: They are larger in size, about 10-12 µm in diameter. Cytoplasm stains deep blue. The nucleus is small, compact and lies peripherally.

It takes 96 hours to form a mature gametocyte. Gametocytes do not develop further inside the red blood cell of man. For further development they must reach the alimentary canal of the female *Anopheles* mosquito. They do not survive for more than a week in human blood if not taken up by the mosquito.

Sexual cycle in female *Anopheles* mosquito

The different stages along with gametocytes of malarial parasite reached the crop (part of alimentary canal) of the mosquito during sucking blood from a man suffering from malaria. In the crop of mosquito all the stages of parasite except the gametocytes are destroyed by the action of enzymes. The sexual cycle of *P. vivax* within mosquito body divides into two phases: (A) Gametogony and (B) Sporogony.

A. Gametogony: The gametogony of *P. vivax* passes following consecutive stages:

1. Formation of male gametes: The male gametes or microgametes are produce from the male gametocyte in the lumen of the crop of mosquito. The nucleus (n) of each male gametocyte mitotically divides to produce 4-8 haploid (n) daughter nuclei. These nuclei move to the periphery of the parasite. At the same time flagella like long threads of cytoplasm push out from the surface of the gametocyte into which a daughter nucleus passes and ultimately dissociating from the original body transformed into a male gamete or microgamete. The process dissociation of male gamete from the rest of the cytoplasm is called **exflagellation.**

2. Formation of female gametes: The female gametes or macrogametes are produce from the female gametocyte in the lumen of the crop of mosquito. With some minor reorganization the female gametocyte (n) becomes a mature female gamete called macrogamete or megagamete. It also forms at its one end a small cytoplasmic projection called **cone of reception** or **cone of fertilization.**

3. Fertilization: An active male gamete (n) penetrates inside of female gamete (n) through the cone of fertilization. The male and female gametes fuse to from a **zygote.** The zygote is diploid (2n).

4. Ookinete: The zygote remains round and motionless for sometime and then transformed into a active elongated form. This active form is called **ookinete** (2n). Each ookinete is 18-24 µm long and 3-5µm wide structure.

5. Oocyst: Each ookinete burrows into and usually through cell of the single layered epithelium of the crop wall and ultimately takes shelter below the basement membrane. By the self made secretion, ookinete becomes surrounded by a thin elastic membrane and by growing is size transforms into a large rounded stage. This large round stage is known as **oocyst (2n)**. The diameter of an oocyst varies from 6μm -60μm. There may be as many as 50-500 oocysts in the crop wall of the female mosquito.

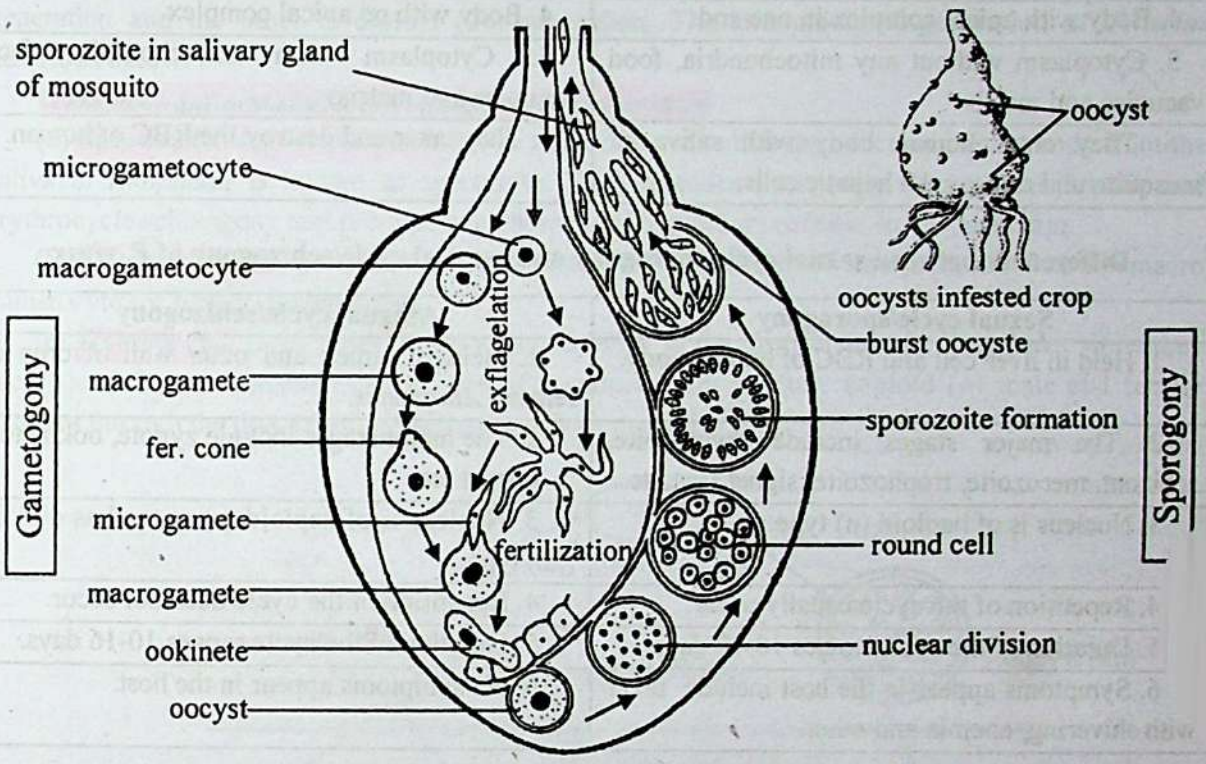


Fig 4.17 Sexual cycle of *P. vivax* in mosquito body

B. Sporogony

The process of formation of sporozoites from the oocyst within the wall crop of mosquito is called **sporogony.** The diploid nucleus (2n) of each oocyst divides first by meiosis (Bano, 1959) and then by mitosis so that a large number of haploid nuclei (n) are formed. Each of the daughter haploid nucleus is surrounded by cytoplasm and converted to small cells. These cells then take the shape of a spindle which is called **sporozoite.** About 10,000 spprozoites are formed from an oocyst. After the maturity of the sporozoites the wall of oocyst breaks down and the sporozoites are liberated in the haemocoel of mosquito. At the end, these sporozoites are deposited inside the salivary gland. The salivary gland of mosquito contains about two lac (200,000) sporozoites. It takes about 10 to 16 days to complete a sexual cycle within *Anopheles* body. During sucking of blood by the mosquito carrying malarial parasites, the sporozoites enter the body of a healthy man through the saliva and starts asexual cycle.

[Some scientists describe sporogony as asexual phage of reproduction. But actually it is an encystement stage of sexual phage oocyst and a continuous process of sexual cycle through which produced active invade able phage of parasite- the sporozoites.]

Differences between sporozoite and trophozoite stage of *P. vivax*

Sporozoite stage	Trophozoite stage
1. Present in salivary glands of *Anopheles*.	1. Present in RBC of human.
2. These are very small, slightly bended spindle shape parasite.	2. These are large and nearly round amoeboid shape parasite.
3. Body covered with elastic pellicle.	3. Body covered with bilayered plasmalema.
4. Body with apical complex in one end.	4. Body with no apical complex.
5. Cytoplasm without any mitochondria, food vacuoles and matrix.	5. Cytoplasm with mitochondria, food vacuoles and matrix.
6. They enter human body with saliva of mosquito and destroy the hepatic cells.	6. They enter and destroy the RBC of human.

Differences between sexual cycle/sporogony and asexual cycle/schizogony of *P. vivax*

Sexual cycle/sporogony	Asexual cycle/schizogony
1. Held in liver cell and RBC of human body.	1. Held in lumen and outer wall of crop of female *Anopheles*.
2. The major stages include cryptozoite, schizont, merozoite, trophozoite, signet ring etc.	2. The major stages include zygote, ookinete oocyst etc.
3. Nucleus is of haploid (n) type.	3. Nucleus is of haploid (n) as well as diploid (2n) type.
4. Repetition of the cycle usually occur.	4. Repetition of the cycle does not occur.
5. Duration of the cycle ranges 10-12 days.	5. Duration of the cycle ranges 10-16 days.
6. Symptoms appear in the host include fever with shivering, anemia and other.	6. No symptoms appear in the host.

Incubation period

Man does not show any disease symptom at once of infection by malarial parasite. The parasite multiplied by repeated schizogony in hepatic cell and RBCs and produced lots of merozoites. Actually the symptoms of malaria are expressed where there are 40-50 merozoites per cubic millimeter of blood are present. **The duration of time between the introduction of sporozoites into the human blood and the first appearance of the symptoms of malarial fever is known as incubation period.** Incubation period varies from species to species. The incubation period of four species of *Plasmodium* are listed below:

Species of malarial parasite	Incubation period (Days)		
	Lowest	Highest	Average
1. *Plasmodium vivax*	12	20	14
2. *Plasmodium ovale*	11	16	14
3. *Plasmodium malariae*	18	40	28
4. *Plasmodium falciparum*	8	15	12

Altérnation of generation of malarial parasite

The regular alternation of forms or of mode of reproduction in the life cycle of an organism, such as the alternation between diploid (2n) and haploid (n) phases, or between sexual and asexual reproductive cycles is called the alternation of generation. This phenomenon is regular in mosses and ferns plant but very rare in animal kingdom except some protozoans. In the life cycle of malarial parasite, *P. vivax* there is an alternation of generation. This parasite reproduces by asexual and sexual processes. There are two kinds of generation in the life cycle. One is haploid (n) generation and the other is diploid (2n) generation. The alternation of generation of *P. vivax* described below:

1. Haploid generation/Asexual haploid phase in man

(a) Sporozoite and Merozoite (n): The stage of parasite that introduce in human body from the saliva of *Anopheles* is known as sporozoite. They reproduce asexually through the hepatic and erythrocycle schizogony and produce huge number of haploid merozoites in blood stream.

(b) Gametocyte (n): Some of the merozoites in blood transformed into haploid micro and macro gametocyte for further development.

(c) Gamete (n): Both types of gametocytes enter in the crop of female *Anopheles* when they suck blood from a malaria infected man. The gametocytes produce haploid (n) male and female gametes through the process of gametogony within mosquito crop.

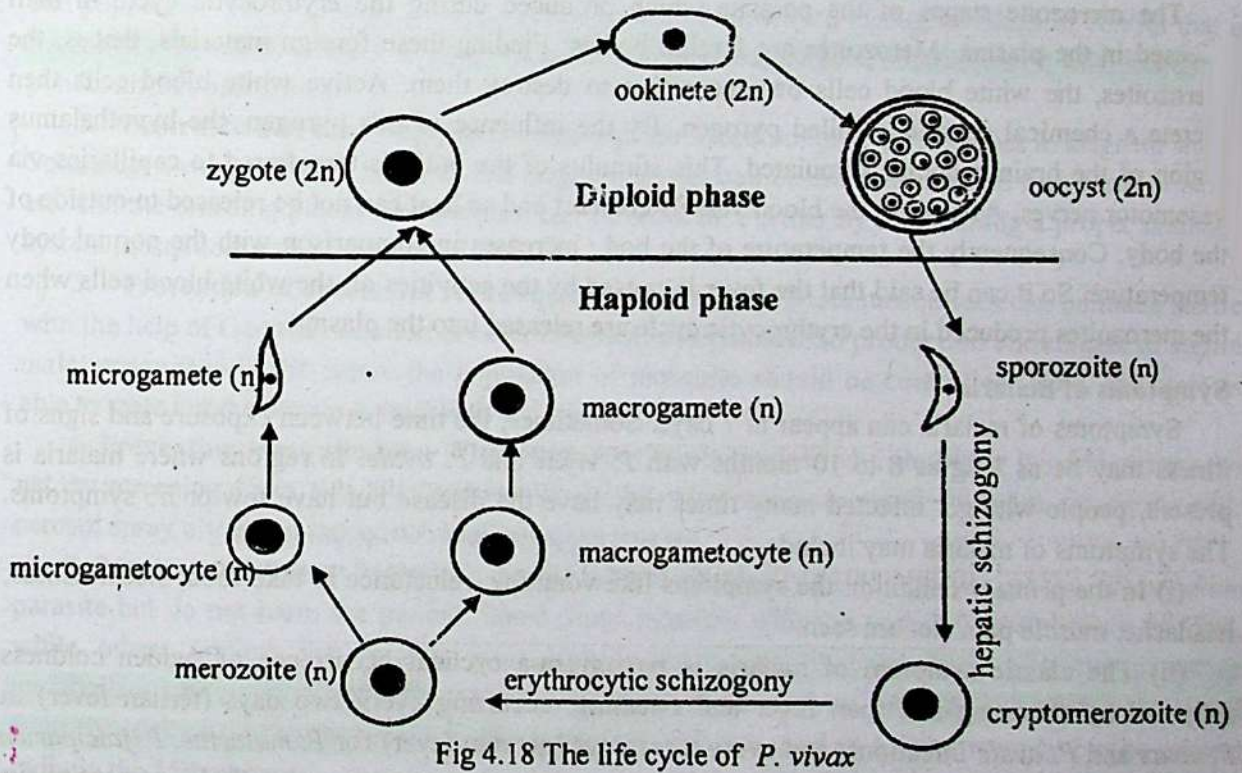


Fig 4.18 The life cycle of *P. vivax*

2. Diploid generation/ Sexual diploid phase in *Anopheles*

(a) Zygote (2n): The male and female gamete fuses to from a **zygote** within crop lumen of mosquito. The zygote is diploid (2n).

(b) Ookinete (2n): The round and motionless zygote transformed into elongated ookinete that take shelter in crop wall.

(c) Oocyst (2n): In course of development ookinete encysted with its own secretion and transformed into a large rounded stage called oocyst. The oocyst produced large number of haploid (n) sporozoites which passes through saliva of mosquito to man where they start asexual phase of life cycle.

It is appears in the life cycle of malarial parasite that the haploid and diploid generations are repeated by turns or consecutively. So in the life cycle of malarial parasite alternation of generation is definitely present.

Significance of alternation of generation:

1. After repeated asexual reproduction by the union of gametes in sexual reproduction the species get new life and regain the necessary power for asexual reproduction.
2. Due to nuclear reorganization in sexual reproduction, organism's abilities to perform physiological activities and the vital power is revived.
3. Alternation of generation is very significant to continue the generation of the species.
4. Alteration of generation can produce variation of the species.
5. Alternation of generation can create diversity in the organisms.

Why there is fever due to the infection of the malarial parasite?

The merozoite stages of the parasite which produced during the erythrocytic cycle in man released in the plasma. Merozoites are foreign bodies. Finding these foreign materials, that is, the merozoites, the white blood cells become active to destroy them. Active white blood cells then secrete a chemical substance called pyrogen. By the influence of this pyrogen, the hypothalamus region of the brain becomes stimulated. This stimulus of the brain is transferred to capillaries via vasomotor nerves. As a result the blood vessels contract and so heat can not be released to outside of the body. Consequently the temperature of the body increases in comparison with the normal body temperature. So it can be said that the fever is caused by the activities of; the white blood cells when the merozoites produced in the erythrocytic cycle are released into the plasma.

Symptoms of malaria

Symptoms of malaria can appear in 7 days. Sometimes, the time between exposure and signs of illness may be as long as 8 to 10 months with *P. vivax* and *P. ovale*. In regions where malaria is present, people who get infected many times may have the disease but have few or no symptoms. The symptoms of malaria may include-

(i) In the primary condition the symptoms like vomiting, reluctance to take food, sleeplessness, headache, muscle pain etc. are seen.

(ii) The classic symptom of malaria is **paroxysm**-a cyclical occurrence of sudden coldness followed by shivering and then fever and sweating, occurring every two days (tertian fever) in *P. vivax* and *P. ovale* infections, and every three days (quartan fever) for *P. malariae*. *P. falciparum* infection can cause recurrent fever every 36–48 hours or a less pronounced and almost continuous fever.

(iii) Severe malaria is usually caused by *P. falciparum* (often referred to as falciparum malaria). Symptoms of falciparium malaria arise 9–30 days after infection. Individuals with cerebral malaria

frequently exhibit neurological symptoms, including abnormal posturing, nystagmus, conjugate gaze palsy (failure of the eyes to turn together in the same direction), opisthotonus, seizures, or coma.

Impact of malaria on RBC/Why does anemia occur in malaria infested man?

(i) Due to the infection of malarial parasite red blood cells are destroyed in a great number. As a result the patient suffers from anemia and the patient becomes pale.

(ii) Spleen and liver enlarges in the patients suffering from malaria for a long time. The liver also secretes a substance called **lysolecithin** which destroys many normal red blood cells.

(iii) The parasite produces an antibody called **haemolysin** which also destroy the normal red blood cells.

Malaria control and prevention

Control of malaria: Malaria can be control by adopting the following measures:

1. Killing of mosquitoes or Vectors: Malarial parasite lives in the liver and blood of man as internal parasite. Female *Anopheles* mosquito carries this parasite from one man to another. So by killing the mosquito, malaria can be controlled. In the following way mosquitoes may be destroyed:

(i) By spraying chemicals adult mosquito can be killed.

(ii) Fumigation of houses by poisonous fumes of Sulpher dioxide (SO_2) kills mosquitoes.

(ii) By spraying BHC, Dieldrin, Paris green, Panama larva killer etc. in the places where larvae and pupae live. The immature stage of mosquito can be destroyed through biological control that is by cultivating different types of fishes like guppy, gambusia, tilapia, smaller carps, khalisha etc. in ponds, ditches and canals

2. Destruction of breeding places of mosquito: Mosquito generally hatches in stagnant water. So useless swampy places, marshes and stagnant waters can be drained or filled with earth or stone etc. and the breeding places for mosquito can be reduced. Further by maintaining a proper drainage system mosquitoes can be controlled.

3. Prevention of successful reproduction in mosquito: Male mosquitoes can be made sterile with the help of Gamma radiation or other chemical compounds. So production and release of sterile male mosquitoes in the nature the population of mosquito should be controlled. Here, the male is able to mate but the female is unable to lay eggs.

4. Preventing mosquito bite: The mosquitoes can be prevented from biting by using mosquito net, by screening doors, windows and ventilators, by using mosquito repellant cream, mosquito coil, aerosol spray ultrasonic mosquito repelling apparatus etc.

5. Medical treatment: Malaria is treated with drugs that block the growth of the *Plasmodium* parasite but do not harm the patient. Some drugs interfere with the parasite's metabolism of food, while others prevent the parasite from reproducing. Drugs that interfere with the parasite's metabolism are related to **quinine,** the first known anti-malarial drug. Quinine is a chemical derived from the bark of the South American **cinchona tree** and was used as a fever remedy by the ancient India in the 15th century.

Chloroquine is a synthetic chemical similar to quinine. It became the drug of choice for malaria when it was developed in the 1940s because it was effective, easy to manufacture, and lacked most of the side effects of quinine.

Mefloquine is another drug related to quinine that is still largely effective, but for many people, especially those living in developing nations, it is too expensive to use routinely.

However, the first choice treatment for malaria is **artemisinin** in combination with **piperaquine.**

Malaria vaccine:

The world's first malaria vaccine has received a green light from European Medicines Agency (EMA) in July 2015. The shot, called **RTS,S** or **Mosquirix,** would be the first licensed human vaccine against a parasitic disease and could help prevent millions of cases of malaria in countries that use it. The vaccine was developed by British drug maker GlaxoSmithKline (GSK) in partnership with the PATH Malaria Vaccine Initiative. Mosquirix, also part-funded by the Bill & Melinda Gates Foundation, will also now be assessed by the World Health Organisation, which has promised to give its guidance on when and where it should be used before the end of this year.

Super malaria

A highly drug-resistant strain of malaria dubbed "**super malaria**" is spreading rapidly in South East Asia, leading scientists to warn that it could soon be a global threat. This dangerous form of the malaria parasite cannot be killed with the main anti-malaria drugs.

The disease, most commonly transmitted by mosquitoes infected with the parasite, was first detected in Cambodia in 2008 but has now spread to the three remaining countries that make up the Eastern Greater Mekong subregion: Vietnam, Laos and Thailand.

In a letter, published in ***Lancet Infectious Diseases***, the team studying the spread of the disease from the Oxford Tropical Medicine Research Unit in Bangkok, said the rapid spread *"presents one of the greatest threats to the control and elimination of malaria."*

The spread of this malaria 'superbug' strain, resistant to the most effective drug we have, is alarming and has major implications for public health globally. Around 700,000 people a year die from drug-resistant infections, including malaria. If nothing is done, this could increase to millions of people every year by 2050.

Did You Know?

- Half of human DNA came from the viruses which inserted into the gametes of our ancestors.
- Viruses can't reproduce by themselves they always take over living cells in plants, and animals.
- Bacteria are the most primitive organisms in the world that exist here since 3.5 billion years.
- Assumed that the power house of cell 'mitochondria' actually evolved from the bacteria that entered the cell billions of years ago and continue as a symbiont within cell.
- Malaria exists in 103 countries worldwide, affecting 3.3. billion people, but about 90% of malaria-related deaths occur in sub-Saharan Africa.
- At least 8 US presidents including George Washington, Abraham Lincoln, John F. Kennedy had malaria.

Exercise

Multiple Choice Questions (Sample)

1. In which organelles of the host cell proteins of virus are synthesized?
   a. Mitochondria b. Ribosome c. Golgi bodies d. Nucleus
2. The causative agent of malaria include in the group of-
   a. Bacteria b. Virus c. Apicomplexa d. Arthropoda